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PROVISIONAL APPLICATION COVER SHEET

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TITLE OF INVENTION (500 characters max)

Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS

CORRESPONDENCE ADDRESS

BIOGEN IDEC / FINNEGAN HENDERSON, LLP **Customer Number 65,779**

ENCLOSED APPLICATION PARTS (check all that apply)

☒ Specification: 40 Pages ☒ Drawing(s): 2 Sheets/2 Figures

☐ Other: [Number] Pages; [Description]

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Respectfully submitted on behalf of the patent practitioners associated with Customer Number 65,779.

SIGNATURE K. Linnik Date February 8, 2007

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☐ Additional inventors are being named on separately numbered sheets attached hereto.

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Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS

[0001] The invention relates to the field of cell and molecular biology and to the development and use of therapeutic compounds, more particularly, compounds for treating neurological diseases, including demyelinating neurological diseases, such as, e.g., multiple sclerosis.

[0002] Multiple sclerosis (MS) is an autoimmune disease with the autoimmune activity directed against central nervous system (CNS) antigens. The disease is characterized by inflammation in parts of the CNS, leading to the loss of the myelin sheathing around neuronal axons (demyelination), loss of axons, and the eventual death of neurons, oligodenrocytes and glial cells.

[0003] An estimated 2,500,000 people in the world suffer from MS. It is one of the most common diseases of the CNS in young adults. MS is a chronic, progressing, disabling disease, which generally strikes its victims some time after adolescence, with diagnosis generally made between 20 and 40 years of age, although onset may occur earlier. The disease is not directly hereditary, although genetic susceptibility plays a part in its development. Relapsing-remitting MS presents in the form of recurrent attacks of focal or multifocal neurologic dysfunction. Attacks may occur, remit, and recur, seemingly randomly over many years. Remission is often incomplete and as one attack follows another, a stepwise downward progression ensues with increasing permanent neurological deficit.

[0004] Although various immunotherapeutic drugs can provide relief in patients with MS, none is capable of reversing disease progression, and some can cause serious adverse effects. Most current therapies for MS are aimed at the reduction of inflammation and suppression or modulation of the immune system. As of 2006, the available treatments for MS reduce inflammation and the number of new episodes but not all have an effect on disease progression. A number of clinical trials have shown that the suppression of inflammation in chronic MS rarely significantly limits the accumulation of disability through

sustained disease progression, suggesting that neuronal damage and inflammation are independent pathologies. Promoting CNS remyelination as a repair mechanism and otherwise preventing axonal loss and neuronal death are some of the important goals for the treatment of MS. For a comprehensive review of MS and its current therapies, see, e.g., McAlpine's Multiple Sclerosis, by Alastair Compston et al., 4th edition, Churchill Livingstone Elsevier, 2006.

[0005] "Phase 2 enzymes" serve as a protection mechanism in mammalian cells against oxygen/nitrogen species (ROS/RNS), electrophiles and xenobiotics. These enzymes are not normally expressed at their maximal levels and, their expression can be induced by a variety of natural and synthetic agents. Nuclear factor E2-related factor 2 (Nrf2) is a transcription factor responsible for the induction of a variety of important antioxidant and detoxification enzymes that coordinate a protective cellular response to metabolic and toxic stress.

[0006] ROS/RNS are most damaging in the brain and neuronal tissue, where they attack post-mitotic (i.e., non-dividing) cells such as glial cells, oligodendocytes, and neurons, which are particularly sensitive to free radicals. This process leads to neuronal damage. Oxidative stress has been implicated in the pathogenesis of a variety of neurodegenerative diseases, including ALS, Alzheimer's disease (AD), and Parkinson's disease (PD). For review, see, e.g., van Muiswinkel et al., Curr. Drug Targets CNS--Neurol. Disord., 2005, 4:267-281. An anti-oxidative enzyme under control of Nrf2, NQO1 (NAD(P)H dehydrogenase, quinone 1), was recently reported to be substantially upregulated in the brain tissues of AD and PD subjects (Muiswinkel et al., Neurobiol. Aging, 2004, 25: 1253). Similarly, increased expression of NQO1 was reported in the ALS subjects' spinal cord (Muiswinkel et al., Curr. Drug Targets--CNS. Neurol. Disord., 2005, 4:267-281) and in active and chronic lesions in the brains of patients suffering from MS (van Horssen et al., Free Radical Biol. & Med., 2006, 41 311-311). These observations indicate that the Nrf2 pathway may be activated in neurodegenerative and neuroinflammatory diseases as an endogenous protective mechanism. Indeed, most recently, it has

been reported that induced activation of Nrf2-dependent genes by certain cyclopenanone-based compounds (NEPP) counters the toxic effects of metabolic inhibition and ROS/RNS production in the brain and protects neurons from death in vitro and in vivo (see Satoh et al., PNAS, 2006, 103(3):768-773).

[0007] Additionally, many publications have reported neuroprotective effects of compounds in natural plant-derived compounds ("phytochemicals"), including α-tocopherol (vitamin E), lycopene (tomatoes), resveratrol (red grapes), sulforaphane (broccoli), EGCG (green tea), etc. For review, see Mattson and Cheng, Trends in Neurosci., 2006, 29(11):632-639. Originally, the action of these compounds was attributed to their anti-oxidant properties. However, while most anti-oxidants are effective only at high concentrations, at least some of these compounds appear to exert neuroprotective effects at much lower doses. Emerging evidence suggests that these compounds may exert their neuroprotective effects by activating cellular stress-response pathways, including the Nrf2 pathway, resulting in the upregulation of neuroprotective genes. However, the exact mechanism of action of these compounds remains poorly understood.

[0008] To date, more than 10 different chemical classes of inducers of Nrf2 pathway have been identified including isothiocyanates and their thiol addition products, dithiocarbamates, as well as 1,2-dithiole-3-thiones, trivalent arsenic derivatives (e.g., phenyl arsenoxide), heavy metals, certain conjugated cyclic and acyclic polyenes (including porphyrins, chlorophyllins, and chlorophyll), and vicinal dimercaptans. These inducers have few structural similarities. They are mostly electrophiles, and all can react chemically with thiol groups by alkylation, oxidation, or reduction, suggesting that the intracellular sensor for inducers is likely to contain very highly reactive (cysteine) thiols. The inducers can modify thiol groups by a variety of mechanisms including: alkylation (Michael addition acceptors, isothiocyanates, quinones); oxidation (e.g., peroxides and hydroperoxides); and direct reaction with thiol/disulfide linkages (e.g., vicinal dithiols such as 1,2-dimercaptopropanol, lipoic acid). These diverse response

mechanisms provide plasticity for cellular responses to a variety of electrophilic and oxidant stressors.

[0009] There is a need to develop new treatments, and in particular, compounds for treating MS that provide neuroprotection. The development of cell-based and in vitro assays to identify and characterize existing drug candidates serves this goal.

[0010] In some embodiments, the invention provides the following methods:

1) methods of screening for new candidate compounds that may be useful for treating a neurological disease;
2) methods of evaluating neuroprotective properties of drugs and drug candidates for treating a neurological disease;
3) methods of comparing (e.g., for bioequivalence) two or more pharmaceutical compositions which contain fumaric acid derivatives;
4) methods of treating a neurological disease by administering to the subject in need thereof compounds that are partially structurally similar to DMF or MMF; and
5) methods of treating a neurological disorder by a combination therapy that includes administration of a first compound that upregulates the Nrf2 pathway and a second compound that does not upregulate the Nrf2 pathway.

[0011] A neurological disease in methods 1-5 above is preferrably a neurodegenerative disease such as, for example, ALS, Parkinson's disease, Alzheimer's disease, and Huntington's disease. More preferably, the neurological disease is MS or another demyelinating neurological disease.

[0012] Methods 1-3 of the invention may comprise:

a) contacting a cell with the test compound, and
b) determining whether the Nrf2 pathway is upregulated in the cell.

In some embodiments, the methods may further comprise:

c) determining whether the test compound slows or prevents demyelination, axonal loss, and/or neuronal death, and/or

d) selecting the test compound as a candidate for treating neurodegeneration in a neurological disease if 1) the Nrf2 pathway is upregulated and 2) demyelination, axonal loss, and/or neuronal death are/is prevented or slowed.

[0013] Methods 1-3 of the invention comprise contacting a cell with test compound(s) and determining whether the Nrf2 pathway is upregulated in the cell. In such methods, an upregulation of the Nrf2 pathway above a threshold (e.g., by at least 30% over a control) indicates that the compound(s) has/have certain biological properties beneficial in treating a neurological disease (e.g., neuroprotective properties). In some embodiments, the upregulation of the Nrf2 pathway is assessed (in vivo and/or in vitro) by one or more of the following:

i) expression levels of endogenously produced or exogenously introduced Nrf2;

ii) subcellular localization and/or nuclear translocation of Nrf2;

iii) expression levels and/or activity of one or more genes under control of Nrf2 (e.g., endogenous NQO1) or an Nrf2-regulated reporter gene in an artificial reporter construct;

iv) levels of Nrf2 binding to the Nrf2-binding DNA element ARE;

v) stability of Nrf2/Keap1 complexes; and

vi) modification (e.g., alkylation) levels of Keap1 and other Nrf2/Keap1-associated proteins.

[0014] In some embodiments of methods 1-3, the compounds that are being screened, evaluated, or compared are mild alkylating agents, and more specifically, Michael addition acceptors, or compounds that are metabolized upon administration to Michael addition acceptors. In some embodiments, such Michael addition acceptors have the structure of Formula I, II, III, or IV set forth in the Detailed Description.

[0015] In certain embodiments of method 1, the method of screening for a candidate compound for treating a neurological disease comprises:

a) contacting a cell with a plurality of test compounds,

b) determining whether the Nrf2 pathway is upregulated in the cell, and

c) selecting from the plurality of compounds at least one compound that upregulates the Nrf2 pathway,

wherein an upregulation of the Nrf2 pathway by the selected compound(s) indicates that the selected compound(s) may be useful for treating a neurological disease. The plurality of compounds may be represented, e.g., by a combinatorial chemical library, and the method may be performed, e.g., by high-throughput screening.

[0016] In certain embodiments of method 2, the method of evaluating neuroprotective properties of a drug or drug candidate for treating a neurological disease comprises:

a) contacting a cell with the drug or drug candidate, and

b) determining whether the Nrf2 pathway is upregulated in the cell,

wherein an upregulation of the Nrf2 pathway by the drug or drug candidate indicates that the drug or drug candidate is useful for neuroprotection in treating a human having a neurological disease.

[0017] In certain embodiments of method 3, a method of comparing two or more pharmaceutical compositions (e.g., for bioequivalence) comprises:

a) contacting a cell with a first composition comprising a test compound, and

b) comparing the level of Nrf2 pathway upregulation in the cell by the test compound to the corresponding level of the Nrf2 pathway upregulation in a control cell treated with a second composition comprising DMF, MMF, or both.

[0018] In some embodiments of method 3, the test compound is fumaric acid, a salt thereof, or a fumaric acid derivative. In some embodiments, the first composition comprises DMF, MMF, or both. In some embodiments, the dose

and/or the formulation of the first composition differs from the dose and/or the formulation of the second composition.

[0019] In some embodiments, method 3 further comprises:

c) comparing at least one pharmacokinetic parameter (e.g., serum-half-life) of the first and the second compositions.

[0020] In some embodiments of method 4, the method of treating a mammal having a neurological disease comprises administering to the mammal a therapeutically effective amount of a neuroprotective compound having Formula I, II, III, or IV, e.g., a fumaric acid derivative (e.g., DMF or MMF).

[0021] In some embodiments of method 4, the invention provides a method of slowing or preventing neurodegeneration (more specifically, e.g., demyelination, axonal loss, and/or neuronal death) in a patient in need thereof, by administering the compound in an amount and for a period of time sufficient to slow or prevent demyelination, axonal loss, and/or neuronal death, e.g., by at least 30% relative to a control.

[0022] In certain embodiments of method 5, the method of treating a mammal having a neurological disease by combination therapy comprises:

a) administering to the mammal a therapeutically effective amount of a first compound that upregulates the Nrf2 pathway, and

b) administering a therapeutically effective amount of a second compound that does not upregulate the Nrf2 pathway.

[0023] In some of embodiments of method 5, the first compound, used in step (a), is a compound of Formula I, II, III, or IV, e.g., a fumaric acid derivative (e.g., DMF or MMF); and the second compound, which is used in step (b), is an immunosuppressive or an immunomodulatory compound that does not upregulate the Nrf2 pathway (e.g., by more than 30% over a control).

[0024] In some embodiments of method 5, the method of treating a neurological disease in a mammal comprises administering to the mammal a therapeutically effective amount of a compound of Formula I, II, III, or IV.

[0025] In some of the embodiments of methods 1-5, the compound being screened, identified, evaluated, or used for treating a neurological disorder is not fumaric acid or its salt, or a fumaric acid derivative (e.g., DMF or MMF).

[0026] Other features and embodiments of the invention will be apparent from the following description and the claims.

BRIEF DESCRIPTION OF THE FIGURES

[0027] **Figure 1** demonstrates that DMF and MMF are potent activators of Nrf2 at concentrations within clinical exposure range (cells in culture).

[0028] **Figure 2** shows results of RNAi experiments.

DETAILED DESCRIPTION

[0029] The present invention is based, in part, on the discovery that dimethyl fumarate (DMF) and monomethyl fumarate (MMF) are potent activators of the Nrf2 pathway, a major neuroprotective and anti-inflammatory mechanism. The invention is further based, at least in part, on the finding that DMF and MMF are neuroprotective (myelinoprotective and axonoprotective) in a mouse model of autoimmune neurodegenerative disease.

[0030] Due to the involvement of Nrf2 in the regulation of cellular response to metabolic stress, survival and inflammation, DMF, MMF, and other Nrf2 activators may be useful for therapeutic management of a variety of inflammatory, ischemic, and neurodegenerative processes.

[0031] Fumaric acid esters, such as DMF, have been proposed for treatment of MS (see, e.g., Schimrigk et al., Eur. J. Neurol., 2006, 13(6):604-10; Drugs R&D, 2005, 6(4):229-30).

[0032] DMF activates a major cytoprotective (neuroprotective) and anti-inflammatory mechanism not targeted by current therapies. Thus, the findings that DMF activates the Nrf2 pathway and has a neuroprotective effect, offer a rationale for the use of DMF in combination with immunosuppressive or

immunodulatory therapeutics that do not upregulate the Nrf2 pathway. The invention further provides means for identifying compounds with a new therapeutic modality useful in multiple neurological indications and complementary to other drugs for the treatment of a neurological disease, including a number of currently used immunomodulators.

[0033] DMF is a member of a large group of anti-oxidant molecules known for their cytoprotective and anti-inflammatory properties. These molecules also share the property of the Nrf2 pathway activation. Thus, the finding that DMF activates the Nrf2 pathway in conjunction with the neuroprotective effects of DMF further offers a rationale for identification of structurally and/or mechanistically related molecules that would be expected to be therapeutically effective for the treatment of neurological disorders, such as, e.g., MS.

I. Definitions

[0034] Certain terms are defined in this section; additional definitions are provided throughout the description.

[0035] The terms "activation" and "upregulation," when used in reference to the Nrf2 pathway, are used interchangeably herein.

[0036] The terms "disease" and "disorder" are used interchangeably herein.

[0037] The term "a drug for treating a neurological disease" refers to a compound that has a therapeutic benefit in a specified neurological disease as shown in at least one animal model of a neurological disease or in human clinical trials for the treatment of a neurological disease.

[0038] The term "neuroprotection" and its cognates refer to prevention or a slowing in neuronal degeneration, including, for example, demyelination and/or axonal loss, and optionally, neuronal and oligodendrocyte death. Neuroprotection may occur through several mechanisms, e.g., through reducing inflammation, providing neurotrophic factors, scavenging free radicals, etc. As used herein, a compound is considered neuroprotective if it (1) upregulates the Nrf2 pathway

above a certain threshold and (2) provides neuroprotection, regardless of possible other mechanisms of action.

[0039] The terms "treatment," "therapeutic method," "therapeutic benefits," and the like refer to therapeutic as well as prophylactic/preventative measures. Thus, those in need of treatment may include individuals already having a specified disease and those who are at risk for acquiring that disease.

[0040] The terms "therapeutically effective dose" and "therapeutically effective amount" refer to that amount of a compound which results in prevention or delay of onset or amelioration of symptoms of a neurological disorder in a subject or an attainment of a desired biological outcome, such as reduced neurodegeneration (e.g., demyelination, axonal loss, and neuronal death) or reduced inflammation of the cells of the CNS.

II. Methods of Evaluating Compounds

[0041] In one aspect, the invention provides method of evaluating neuroprotective properties of test compounds, including the following methods:

1) methods of screening for new candidate compounds that may be useful for treating a neurological disease;
2) methods of evaluating neuroprotective properties of drugs and candidates that are used or proposed for treating a neurological disease;
3) methods of comparing (e.g., for bioequivalence) two or more pharmaceutical compositions which contain fumaric acid derivatives;

[0042] Methods 1-3 of the invention may comprise:

a) contacting a cell with the test compound,

b) determining whether the Nrf2 pathway is upregulated in the cell,

and, in some embodiments, additionally performing the following step(s):

c) determining whether the test compound slows or prevents demyelination, axonal loss, and/or neuronal death, and/or

d) selecting the test compound as a candidate for treating neurodegeneration in a neurological disease if 1) the Nrf2 pathway is upregulated and 2) demyelination, axonal loss, and/or neuronal death are/is prevented or slowed.

[0043] Methods 1-3 are described in detail below.

Method 1: Methods of screening

[0044] The invention provides methods of screening for a candidate compound for treating a neurological disease. Such methods comprise:

a) contacting a cell with a plurality of test compounds,
b) determining whether the Nrf2 pathway is upregulated in the cell, and
c) selecting from the plurality of compounds at least one compound that upregulates the Nrf2 pathway,

wherein an upregulation of the Nrf2 pathway by the selected compound(s) indicates that the selected compound(s) may be useful for treating a neurological disease. For example, the plurality of compounds may be represented by a combinatorial chemical library, and the screening method may be performed by a high-throughput screening as described in, e.g., High-Throughput Screening in Drug Discovery (Methods and Principles in Medicinal Chemistry), by Jörg Hüser (ed.), John Wiley & Sons (2006).

[0045] Combinatorial libraries of compounds are also described in, e.g., Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries (Tetrahedron Organic Chemistry) Ian Salusbury (ed.), Elsevier (1998); Combinatorial Libraries: Synthesis, Screening and Application Potential (Library Binding), by Riccardo Cortese (ed.), Walter de Gruyter (1995). The libraries of compounds may be, for example, quinone libraries and other libraries as described in Mittoo, Comb. Chem. & High Throughput Screen, 2006, 9:421-423.

[0046] In some embodiments, the compounds that are being screened and/or selected comprise at least one or a plurality of mild alkylating agents, and

more particularly, Michael addition acceptors or compounds that are metabolized to Michael addition acceptors, including compounds of Formulas I, II, III, or IV.

[0047] In some of the embodiments, the compounds comprise fumaric acid, its salt(s), and/or fumaric acid derivative(s).

Methods 2: Methods of evaluating drugs and drug candidates

[0048] The invention further provides methods of evaluating neuroprotective properties of a drug or drug candidate for treating a neurological disease. Such methods comprise:

a) contacting a cell with the drug or drug candidate, and

b) determining whether the Nrf2 pathway is upregulated in the cell, wherein the upregulation of the Nrf2 pathway by the drug or drug candidate indicates that the drug or drug candidate is neuroprotective in treating a human having a neurological disease.

[0049] In some embodiments, the upregulation of the Nrf2 pathway by the drug or drug candidate indicates that the drug or drug candidate has the ability to slow demyelination, the loss of axons, and/or neuronal death.

[0050] In some embodiments, the method of evaluating a drug or drug candidate comprise an additional step:

c) evaluating demyelination, loss of axons, and/or neuronal death.

[0051] In some embodiments, steps a) and c) are performed in vivo in at least one model of a neurological disease, e.g., as described below.

[0052] In other embodiments, particularly those in which the neurological disease is multiple sclerosis or another demyelinating disease, the evaluated drug or drug candidate for a neurological disease is chosen from the following: FTY720 (2-(4-octylphenethyl)-2-aminopropane-1,3-diol; Novartis); anti-IL12 antibody (e.g., ABT-874; Abbott Laboratories); GSK683699 (GSK/Tanabe); NeuroVax (Immune Response Corp.; Darlington, Curr. Opin. Mol. Ther., 2005, 7(6):598-603); anti-CCR2 antibody (e.g., MLN 1202; Millennium); interferon β-1a (e.g., Avonex®; Biogen Idec); anti-α4-integrin antibody (e.g., Tysabri®; Biogen Idec/Elan); anti-CD20 antibody (e.g., Rituxan® (Biogen Idec/Genentech);

TV 5010 (Teva); NBI-788 (Neurocrine); MBP8298 (BioMS (see Warren et al., Eur. J. Neurol., 2006, 13(8):887-95); Mylinax (Oral Cladribine; 2-chlorodeoxyadenosine; Serono/IVAX); Teriflunomide ((Z)-2-cyano-N-(4-(trifluoromethyl)phenyl)-3-hydroxybut-2-enamide; Sanofi-Aventis); Temsirolimus (Wyeth); Laquinimod (5-chloro-N-ethyl-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-N-phenylquinoline-3-carboxamide; Active Biotech/Teva); and interferon tau (Tauferon; Pepgen).

[0053] In some embodiments, the drug or drug candidate being evaluated is a mild alkylating agent, and more specifically, a Michael addition acceptor, or a compound that is metabolized to a Michael addition acceptor, including compounds of Formulas I, II, III, or IV.

[0054] In some of the embodiments, the compound is fumaric acid, its salt, or a fumaric acid derivative.

Method 3: Methods of methods of evaluating neuroprotective properties

[0055] The invention further provides methods of comparing (e.g., for bioequivalence) two or more pharmaceutical compositions. Such methods comprise:

a) contacting a cell with a first composition comprising a test compound, and

b) comparing the level of the Nrf2 pathway upregulation in the cell by the test compound to the corresponding level of the Nrf2 pathway upregulation in a cell treated with a second composition ("comparator composition") comprising DMF, MMF, or both.

[0056] In some embodiments, substantially dissimilar levels of upregulation by the first and second compositions indicate that the compositions are not bioequivalent.

[0057] In some embodiments, the test compound is fumaric acid, its salt thereof, a fumaric acid derivative, or mixtures thereof. In some embodiments, the first composition comprises DMF, MMF, or both. In some embodiments, the dose and/or the formulation of the first composition differs from the dose and/or

the formulation of the second composition. The first composition may be a controlled release composition such as, e.g., compositions described in WO 2006/037342.

[0058] In some embodiments, the method further comprises and additional step:

c) comparing at least one pharmacokinetic parameter of the first and the second compositions.

[0059] Pharmacokinetic parameters and methods for evaluating the same are well known and are described in, e.g., Pharmacokinetics, Second Edition (Drugs and the Pharmaceutical Sciences) by Milo Gibaldi et al. (eds.), Informa Healthcare (1982). Examples of such pharmacokinetic parameters that can be evaluated include serum half-life, clearance, and volume distribution.

[0060] In some embodiments, substantially dissimilar pharmacokinetic parameter(s) of the first and second compositions indicate that the compositions are not bioequivalent.

[0061] In some embodiments, the test compound being evaluated is a mild alkylating agent, and more specifically, a Michael addition acceptor, or a compound that is metabolized to a Michael addition acceptor.

[0062] In some of the embodiments, the test compound is fumaric acid or its salt, or a fumaric acid derivative.

III. Methods of Treatment

[0063] The invention provides methods of treating a mammal who has or is at risk for developing a neurological disease, including the following methods:

4) methods of treating a neurological disease by administering to the subject in need thereof compounds that are partially structurally similar to DMF or MMF (including compounds selected using methods 1-3 described above) ; and

5) methods of treating a neurological disorder by a combination therapy that includes administration of a first compound that does

not upregulate the Nrf2 pathway and a second compound that upregulates the Nrf2 pathway.

[0064] Methods 4-5 are described in detail below.

Method 4: Treatment methods

[0065] The invention further provides methods of treating a neurological disease by administering to the subject in need thereof compounds that are partially structurally similar to DMF or MMF.

[0066] In some embodiments of method 4, the invention provides a method of treating a mammal who has or is at risk for a neurological disease. The method comprises administering to a mammal a therapeutically effective amount of a neuroprotective compound which has Formula I, II, III, or IV, e.g., a fumaric acid derivative (e.g., DMF or MMF).

[0067] In some embodiments of method 4, the invention provides a method of slowing or preventing neurodegeneration (more specifically, e.g., demyelination, axonal loss, and/or neuronal death) in a subject in need thereof, by administering the compound in an amount and for a period of time sufficient to slow or prevent demyelination, axonal loss, and/or neuronal death, e.g., by at least 30%, 50%, 100% or higher over a control over a period of at least 5, 10, 12, 20, 40, 52, 100, or 200 weeks.

Method 5: Combination therapy

[0068] The methods of treatment further include methods of treating a mammal having a neurological disease by combination therapy. Such methods comprise:

a) administering to the mammal a therapeutically effective amount of a first compound that upregulates the Nrf2 pathway, and
b) administering a therapeutically effective amount of a second compound that does not upregulate the Nrf2 pathway.

[0069] In some of embodiments of method 5, the first compound, used in step (a), is a compound of Formula I, II, III, or IV, e.g., DMF or MMF; and the second compound, which is used in step (b), is an immunosuppressive or an

immunomodulatory compound that does not upregulate the Nrf2 pathway (e.g., by more than 30%, 50%, 100% over a control).

[0070] In some embodiments of method 5, the method of treating a neurological disease in a mammal, comprises administering to the mammal a therapeutically effective amount of a compound of Formula I, II, III, or IV.

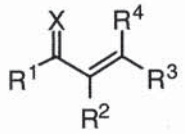
[0071] In method 5, the first compound and the second compound may be administered concurrently (as separate compositions or a mixed composition) or consecutively over overlapping or non-overlapping intervals. In the sequential administration, the first compound and the second compound can be administered in any order. In some embodiments, the length of an overlapping interval is more than 2, 4, 6, 12, 24, or 48 weeks.

IV. Michael addition acceptors

[0072] Michael addition acceptors generally include olefins or acetylenes conjugated to an electron withdrawing group, such as carbonyl containing groups, thiocarbonyl containing groups, cyano, sulfonyl, sulfonamido, amido, formyl, keto, and nitro. Exemplary carbonyl groups include carboxylic acid esters and carboxylic acid.

[0073] In some embodiments of methods 1-5, the compound(s) that are being screened, identified, evaluated, or used for treating a neurological disorder are mild alkylating agent(s), and more specifically, Michael addition acceptor(s), or compound(s) that are/is metabolized to Michael addition acceptor(s).

[0074] In some embodiments, the Michael addition acceptor(s) have/has the structure of Formula I:



(I)

wherein:

X is O; S; C(R)(C_{1-12})alkyl; or C(R)(C_{2-12})alkenyl, wherein R is H, (C_{1-12})alkyl or (C_{2-12})alkenyl;

R^1, R^2, R^3 and R^4 are independently selected from the group consisting of: H; OH; O^-; CO_2H, CO_2^-; SH; S^-; SO_2H, SO_2^-; (C_{1-24})alkyl; (C_{1-24})alkenyl; (C_{6-50})aryl, $CO_2(C_{1-24})$alkyl; $SO_2(C_{1-24})$alkyl, $CO_2(C_{1-24})$alkenyl; $SO_2(C_{1-24})$alkenyl; CO_2Y, wherein Y is psoralen-9-yl, retinyl, alpha-tocopherol, calciferyl, corticostreoid-21-yl or monosaccarid-ω-yl; (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; (C_{6-50})aryloxy; (C_{1-24})alkylthio; (C_{1-24})alkenylthio; (C_{6-50})arylthio, amino; amido; arylalkyl; cyano; nitro; sulfonyl, sulfoxido, sulfonamido; formyl, keto; or D or L natural or unnatural amino acids; or any two of X, R^1, R^2 and R^3, and R^4 may be joined together to form a cyclic moiety; and wherein the alkyl, alkoxy, alkenyl, alkenyloxy, aryl and aryloxy groups may be optionally substituted with at least one of halogen (F, Cl, Br, I), OH, (C_{1-4})alkoxy, nitro or cyano; or

a pharmaceutically acceptable salt thereof.

[0075] In some embodiments, the Michael addition acceptor(s) have/has the structure of Formula I, with the following provisos:

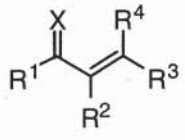
R^1 is selected from: H; OH; O^-; CO_2H, CO_2^-; SH; S^-; SO_2H, SO_2^-; (C_{1-24})alkyl; (C_{1-24})alkenyl; (C_{6-50})aryl, $CO_2(C_{1-24})$alkyl; $SO_2(C_{1-24})$alkyl, $CO_2(C_{1-24})$alkenyl; $SO_2(C_{1-24})$alkenyl; CO_2Y, wherein Y is psoralen-9-yl, retinyl, alpha-tocopherol, calciferyl, corticostreoid-21-yl or monosaccarid-ω-yl; (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; (C_{6-50})aryloxy; (C_{1-24})alkylthio; (C_{1-24})alkenylthio; (C_{6-50})arylthio, arylalkyl, amino; amido; cyano; nitro; sulfonyl, sulfoxido, sulfonamido; formyl, keto; or D or L natural or unnatural amino acids; and wherein the alkyl, alkoxy, alkenyl, alkyenyloxy, aryl and aryloxy groups may be optionally substituted with at least one of halogen (F, Cl, Br, I), OH, (C_{1-4})alkoxy, nitro or cyano;

R^2 is selected from: H; CO_2H, CO_2^-; SO_2H, SO_2^-; (C_{1-24})alkyl; (C_{1-24})alkenyl; (C_{6-50})aryl, $CO_2(C_{1-24})$alkyl; $SO_2(C_{1-24})$alkyl, $CO_2(C_{1-24})$alkenyl; $SO_2(C_{1-24})$alkenyl; CO_2Y, wherein Y is psoralen-9-yl, retinyl, alpha-tocopherol,

calciferyl, corticostreoid-21-yl or monosaccarid-ω-yl; (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; (C_{6-50})aryloxy; (C_{1-24})alkylthio; (C_{1-24})alkenylthio; (C_{6-50})arylthio, amido; arylalkyl; cyano; nitro; sulfonyl, sulfoxido, sulfonamido; formyl, keto; or D or L natural or unnatural amino acids; wherein the alkyl, alkoxy, alkenyl, alkyenyloxy, aryl and aryloxy groups may be optionally substituted with at least one of halogen (F, Cl, Br, I), OH, (C_{1-4})alkoxy, nitro or cyano; and

R^3 and R^4 are independently selected from: H; CO_2H, CO_2^-; SO_2H, SO_2^-; (C_{1-24})alkyl; (C_{1-24})alkenyl; (C_{6-50})aryl, CO_2(C_{1-24})alkyl; SO_2(C_{1-24})alkyl, CO_2(C_{1-24})alkenyl; SO_2(C_{1-24})alkenyl; CO_2Y, wherein Y is psoralen-9-yl, retinyl, alpha-tocopherol, calciferyl, corticostreoid-21-yl or monosaccarid-ω-yl; (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; (C_{6-50})aryloxy; (C_{1-24})alkylthio; (C_{1-24})alkenylthio; (C_{6-50})arylthio, amido; arylalkyl; cyano; nitro; cyano; nitro; sulfonyl, sulfoxido, sulfonamido; formyl, keto; wherein the alkyl, alkoxy, alkenyl, alkyenyloxy, aryl and aryloxy groups may be optionally substituted with at least one of halogen (F, Cl, Br, I), OH, (C_{1-4})alkoxy, nitro or cyano.

[0076] In some embodiments, the Michael addition acceptor(s) have/has the structure of Formula II:



(II)

wherein:

X is O or S or C(R)(C_{1-12})alkyl; or C(R)(C_{2-12})alkenyl, wherein R is H, (C_{1-12})alkyl or (C_{2-12})alkenyl;

R^1, R^2, R^3, and R^4 are independently selected from the group consisting of: H; OH; O^-; CO_2H, CO_2^-; (C_{1-12})alkyl; (C_{1-12})alkenyl; CO_2(C_{1-12})alkyl; or any two of X, R^1, R^2 and R^3 may be joined together to form a cyclic moiety;

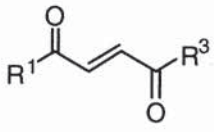
or a pharmaceutically acceptable salt thereof.

[0077] In some embodiments of the compounds of Formulae I-IV, the pharmaceutically acceptable salt is a salt of a metal (M) cation, wherein M can be an alkali, alkaline earth, or transition metal such as Li, Na, K, Ca, Zn, Sr, Mg, Fe, or Mn.

V. Fumaric acid derivatives

[0078] In some embodiments of methods 1-5, the compounds of Formula I include fumaric acid, its salts, and fumaric acid derivatives.

[0079] In some embodiments, the compounds of Formula I have the structure of Formula III:



(III),

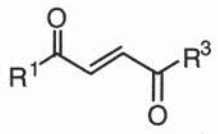
wherein:

R^1 and R^3 are independently selected from OH; O^-, (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; (C_{6-50})aryloxy; psoralen-9-yloxy, retinyloxy, alpha-tocopheroloxy, calciferyloxy, corticostreoid-21-yloxy, monosaccarid-ω-yloxy; amino, or a D or L natural or unnatural amino acid; and wherein the (C_{1-24})alkoxy; (C_{1-24})alkenyloxy; or (C_{6-50})aryloxy groups may be optionally substituted with at least one of halogen (F, Cl, Br, I), OH, (C_{1-4})alkoxy, nitro or cyano;

or a pharmaceutically acceptable salt thereof.

[0080] Compounds wherein at least one of R^1 and R^3 is derived from a natural or unnatural D or L amino acid are described in U.S. Application Serial Nos. 10/433,295, paragraphs 10 to 11 and 18-28, and 11/421,083, which are incorporated herein by reference.

[0081] In some embodiments, the compound of formula (I) have the structure of Formula IV:



(IV),

wherein:

R^1 and R^3 are independently selected from OH; O^-, (C_{1-24})alkoxy, allyloxy, vinyloxy, (C_{6-50})aryloxy; psoralen-9-yloxy, retinyloxy, alpha-tocopheroloxy, calciferyloxy, corticostreoid-21-yloxy, monosaccarid-ω-yloxy; amino, or a D or L natural or unnatural amino acid; and wherein the (C_{1-24})alkoxy, allyloxy, vinyloxy, or (C_{6-50})aryloxy may be optionally substituted with at least one of Cl, F, I, Br, OH, (C_{1-4})alkoxy, nitro, or cyano;

or a pharmaceutically acceptable salt thereof.

[0082] In some embodiments, the "fumaric acid derivative" is chosen from the compounds of Formula III, compounds of Formula IV and the following:

1) fumaric acid amides derived from natural and unnatural amino D or L acids, as described in U.S. Patent Application Serial Nos. 10/433,295, paragraphs 10 to 11 and 18-28, and 11/421,083.

2) a carbocyclic or oxacyclic fumaric acid oligomer as described in U.S. Patent Application Serial No. 10/511,564, paragraphs 15-44; and

3) a glycerol or alkane diol or polyol derivative of fumaric acid as described in U.S. Patents Nos. 4,851,439, 5,149,695, 5,451,667, at cols. 2-4.

[0083] In some embodiments, "fumaric acid derivative" is a dialkyl fumarate (e.g., DMF), and mono alkyl fumarates (MMF) and their salts.

[0084] In some of the embodiments of methods 1-5, the compound being screened, evaluated, compared or used for treating a neurological disorder is not fumaric acid or its salt, or a fumaric acid derivative (e.g., DMF or MMF).

VI. Nrf2 pathway and Nrf2-regulated genes

[0085] Nrf2 (Nuclear Factor-E2-related factor 2; for sequence of the Nrf2, see Accession No. AAB32188) is the transcription factor that, upon activation by

oxidative stress, binds to the antioxidant response element (ARE), and activates transcription of Nrf2-regulated genes. This pathway has been well characterized for its role in hepatic detoxification and chemoprevention through the activation of phase II gene expression. ARE-regulated genes may also contribute to the maintenance of redox homeostasis by serving as endogenous anti-oxidant systems. At present, the list of Nfr2-regulated genes contains over 200 genes encoding proteins and enzymes involved in detoxification and antioxidant response (Kwak et al., J. Biol. Chem., 2003, 278:8135) such as, e.g., HO-1, ferritin, glutathione peroxidase, glutathione-S-transferases (GSTs), NAD(P)H:quinone oxidoreductases, now commonly known as nicotinamide quinone oxidoreductase 1 (NQO1; EC 1.6.99.2; also known as DT diaphorase and menadione reductase), NQO2, g-glutamylcysteine synthase (g-GCS), glucuronosyltransferase, ferritin, and heme oxygenase-1 (HO-1), as well as any one of the enzymes proteins listed in Table 1 in Chen & Kunsch, Curr. Pharm. Designs, 2004, 10:879-891; Lee et al., J. Biol. Chem., 2003, 278(14):12029-38, and Kwak, supra.

[0086] Accordingly, in some embodiments, the Nrf2-regulated gene which is used to assess the activation of the Nrf2 pathway is a phase II detoxification enzyme, an anti-oxidant enzyme, an enzyme of the NADPH generating system, and/or Nrf2 itself. Examples of the phase II detoxification enzymes include NQO1, NQO2, GST-Ya, GST-pi, GST-theta 2, GST-mu (1,2,3), microsomal GST 3, catalytic y-GCS, regulatory-GCS, microsomal epoxide hydrolase, UDP-glucuronosyltransferase, transaldolase, transketolase, and drug-metabolizing enzyme. Examples of the anti-oxidant enzymes include HO-1, ferritin (L), glutathione reductase, glutathione peroxidase, metallothionein I, thioredoxin, thioredoxin reductase, peroxiredoxin MSP23, Cu/Zn superoxide dismutase, and catalase. Examples of the enzymes of the NADPH generating system include malic enzyme, UDP-glucose dehydrogenase, malate oxidoreductase, and glucose-6-phosphate dehydrogenase.

[0087] The antioxidant response element (ARE, also referred to as the electrophile response element (EpRE), GRE1, ARE4, and StREb) is a *cis*-acting DNA regulatory element with a core nucleotide sequence of 5'-TGA(C/T/G)NNNGC-3' (SEQ ID NO:1) (Rushmore et al., J. Biol. Chem., 1991, 266(18):11632-9; see also Nioi et al., Mutation Res., 2004, 555:14-171).

[0088] Accordingly, in some embodiments, the sequence of the DNA element ARE element, to which Nrf2 binds (whether the former is a part of an endogenous gene or an artificial construct), comprises the core ARE sequence TGA(C/T/G)NNNGC (SEQ ID NO:2) or the ARE consensus sequence (G/A)TGA(C/T/G)NNNGC(A/G) (SEQ ID NO:3). In further specific embodiments, the ARE sequence comprises any one of the specific "minimal enhancer" sequences shown in Table 1.

[0089] In some embodiments, the ARE sequence further comprises corresponding 5'- and 3'-USR sequences as shown in Table 1. In some embodiments, the ARE sequence comprises the sequence GTGANNNNGCA (SEQ ID NO:4), or more particularly, the mouse (NNNN=gtcg) or human (NNNN=ctca) versions thereof.

Table 1

Species	Gene	Element	5'-USR	Minimal enhancer	3'-USR	SEQ ID NO
mouse	nqo1	ARE	agTCAca	GTGAgtcgGCA	aaattt	SEQ ID NO:5
rat	NQO1	ARE	agTCAca	GTGACttgGCA	aaatct	SEQ ID NO:6
human	NQO1	ARE	agTCAca	GTGACtcaGCA	gaatct	SEQ ID NO:7
mouse	gsta1	EpRE	gcTAAtg	GTGACaaaGCA	actttc	SEQ ID NO:8
rat	GSTA2	ARE	gcTAAtg	GTGACaaaGCA	actttc	SEQ ID NO:9
mouse	gsta3	ARE	ctcAggc	ATGACattGCA	tttttc	SEQ ID NO:10
rat	GSTP1	GPE1	agTCAct	ATGATtcaGCA	acaaaa	SEQ ID NO:11
human	GCLC	ARE4	ccTCccc	GTGACtcaGCG	ctttgt	SEQ ID NO:12
human	GCLM	EpRE	gaagAca	ATGACtaaGCA	gaaatc	SEQ ID NO:13
mouse	ho1	StREb	cccAAcc	ATGACacaGCA	taaaag	SEQ ID NO:14
ARE 'core'...				TGACnnnGC		SEQ ID NO:15
ARE consensus ...				TAAnn ATGACnnnGCA C G T G	aaaa tttt	SED ID NO:16

[0090] Under basal conditions, Nrf2 is sequestered in the cytoplasm to the actin-bound Kelch-like ECH-associated protein 1 (Keap1; Accession No. NP_987096 for human Keap1), a Cullin3 ubiquitin ligase adaptor protein. More specifically, the N-terminal domain of Nrf2, known as Neh2 domain, is thought to interact with the C-terminal Kelch-like domain of Keap1. In response to xenobiotics or oxidative stress, Nrf2 is released from the Keap1/Nrf2 complex, thereby promoting nuclear translocation of Nrf2 and concomitant activation of ARE-mediated gene transcription. Keap1 function, in turn, requires association with Cullin3, a scaffold protein that positions Keap1 and its substrate in proximity

to the E3 ligase Rbx1, allowing the substrate (Nrf2) to be polyubiquitinated and thus targeted for degradation. The exact mechanism of how the Keap1/Nrf2 complex senses oxidative stress remains poorly understood. Human Keap1 contains 25 cysteine residues that were hypothesized to function as sensors of oxidative stress; 9 of the cysteines are thought to be highly reactive (Dinkova-Kostova et al., PNAS, 2005, 102(12):4584-9). It was theorized but is not relied on for the purposes of this invention that alkylation of cysteins leads to a conformational change, resulting in the liberation of Nrf2 from Nrf2/Keap1/Cullin3 complexes, followed by nuclear translocation of the liberated Nrf2.

VII. Assays for determining the Nrf2 pathway activation

[0091] Methods 1-3 of the invention comprise contacting a cell with test compound(s) and determining whether the Nrf2 pathway is upregulated in the cell. In such methods, an upregulation of the Nrf2 pathway above a threshold (e.g., by at least 30%, 50%, 100%, 200%, 500% over a control) indicates that the compound(s) has/have certain biological properties beneficial in treating a neurological disease (e.g., neuroprotective properties).

[0092] The ability of compound to activate the Nrf2 pathway can be determined by one or more in vitro and in vivo assays, including, e.g., the following assays described below.

[0093] i) *Expression levels of Nrf2 of endogenously or exogenously introduced produced Nrf2*--The sequence of the promoter region of the *nrf2* gene (–1065 to –35) has been published, for example, in Chan et al., PNAS, 1996, 93:13943-13948. One may use an artificially constructed expression construct containing the Nrf2 promoter element and an artificial reporter gene. Alternatively, one may use PCR or Northern blotting to determine expression levels of Nrf2 mRNA, or Western blotting to determine Nrf2 protein levels. Exemplary procedures for determining expression levels of Nrf2 are described in Kwak et al., Mol. Cell. Biol. 2002, 22(9):2883-2892 and Kwak et al., Mol. Med., 2001, 7:135-145. Antibodies against Nrf2 are can be produced by methods

known in the art and are commercially available from, for example, StressGen. Accordingly, in some embodiments, the Nrf2 pathway is activated so that the expression levels of Nrf2 are increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

[0094] ii) *Subcellular localization and/or nuclear translocation of Nrf2*--Such assays include cell staining, or analysis of cytoplasmic versus nuclear cell extracts. For example, a Nrf2-green fluorescence protein (GFP) fusion protein construct can be made and introduced into cells and visualized as described in, e.g., Kraft et al., J. Neurosci., 2004, 24, 1101-1112; and Satoh et al., PNAS, 2006, 103(3):768-773. Accordingly, in some embodiments, the Nrf2 pathway is activated so that the ratio between cytomplasmic and nuclear Nrf2 is elevated by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

[0095] iii) *Expression levels and/or activity of one or more genes under the control of Nrf2*--Such genes under the control of Nrf2 include endogenous or artificially introduced reporter genes in reporter constructs introduced into cells. For example, expression levels of endogenous or exogenously introduced NQO1 may be determined as described in the Examples. Alternatively, a reporter gene construct with one or more ARE sites operably linked to a reporter gene (e.g., luceferase or GFP) can be made, as described in, e.g., Satoh et al., PNAS, 2006, 103(3):768-773. Expression levels of an Nrf-2 induced gene product can be measured at the protein (e.g., by Western blotting or enzymatic activity assays) or at the mRNA levels (e.g., by PCR). Methods for performing RT-PCT are described in, e.g., Calabrese et al., J. Neurosci. Res., 2005, 79:509-521 for HO-1, in Wierinckx et al., J. Neuroimmunology, 2005, 166:132-143 for NQO1. Methods for measuring enzymatic activity of NQO1, using for example, menadione as a substrate, are described in Dinkova-Kostova et al., PNAS, 2001, 98:3404-09 or by Prochaska et al., Anal. Biochem., 1988, 169:328-336. Methods for measuring GST activity, using for example, 1-chloro-2,4-dinitrobenzene as a substrate, are described in Ramos-Gomez et al., J. Neurosci., 2004,

24(5):1101-1112 and Habig et al., 1974, J. Biol. Chem., 219, 7130-7139. Methods for measuring HO-1 activity are described in, e.g., in Calabrese et al., 2005, J. Neurosci. Res., 79:509-521. Accordingly, in some embodiments, the Nrf2 pathway is activated so that the expression levels and/or activity of the gene produced are increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

[0096] iv) *Levels of Nrf2 binding to ARE*--For example, such assays may utilize electromobility shift assays (EMSA) and Chromatin Immununoprecipitation (ChIP) assay, as described in, e.g., Satoh et al., PNAS, 2006, 103(3):768-773 and Kwak et al., Mol. Cell Biol., 2002, 22(9):2883-2892. Accordingly, in some embodiments, the Nrf2 pathway is activated so that the level of Nrf2 binding to ARE is increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

[0097] v) *The stability of Nrf2/Keap1 complexes*--Such assay may include analysis of immunoprecipitated complexes with Nrf2 and/or Keap1 or other Nrf2/Keap1-associated proteins as described in, e.g., Satoh et al., PNAS, 2006, 103(3):768-773. Anti-Keap1 antibodies can be produced using methods known in the art and are available commercially from, for example, Santa Cruz Biotechnology. Accordingly, in some embodiments, the Nrf-2 pathway is activated so that the stability of Nrf2/Keap1 complexes is increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

[0098] vi) *Modification (e.g., alkylation levels) of Keap1 and other Nrf2/Keap1-associated proteins*--Such assays may include mass spectrometric analysis of immunoprecipitated Keap1, using techniques as described in, e.g., Dinkova-Kostova et al., PNAS, 2005, 102(12):4584-9 and Gao et al., J. Biol. Chem., on-line pub. Manuscript M607622200. In some embodiments, the Nrf-2 pathway is activated so that the level of Keap1 and other Nrf2/Keap1-associated proteins is increased by, for example, at least 30%, 50%, 100%, 200%, 500% or more as compared to the non-activated state.

[0099] Alkylating capacity of a compound can be assessed using recombinant Keap1, by a competition reaction with 5,5'-dithiobis(2-nitrobezoic acid) (DTNB) as described in, e.g., Gao et al., J. Biol. Chem., on-line pub. Manuscript M607622200.

[0100] In some embodiments, the cell being contacted with test compound(s) is a neuron or a neuronal cell line. In specific embodiments, the cell being contacted with test compound(s) is a colon carcinoma cell line (e.g., DLD1), the neuroblastoma cell lines (e.g., SkNSH or IMR32), and a primary monocyte. The cell may be a cell in culture (in vitro) or be inside of an animal (in vivo methods).

[0101] Cell viability, and in particular, neuronal viability can be assessed in vivo or in vitro using any suitable method, including methods as described in the Examples. For example, neuronal viability can be assessed using an MTT assay after exposure of neuronal cell cultures to cytotoxic levels of glutamate as described in, e.g., Shih et al., J. Neurosci., 2005, 25(44):10321-35. Additionally, cell viability may also be assessed in assays in which cell death is induced by oxidative damage, for example, by the addition of glucose oxidase to astrocyte cell cultures, as described in, e.g., Calabrese et al., J. Neurosci. Res., 2005, 79:509-521. In vivo assays may be performed as described in, e.g., Misgeld, Histochem. Cell Biol., 2005, 124:189-196.

[0102] The amount of the reporter gene expressed can be determined by any suitable method. Expression levels, at the RNA or the protein level, can be determined using routine methods. Expression levels are usually scaled and/or normalized per total amount of RNA or protein in the sample and/or a control, which is typically a housekeeping gene such as actin or GAPDH. RNA levels are determined by quantitative PCR (e.g., RT-PCR), Northern blotting, or any other method for determining RNA levels, e.g., as described in Cloning: A Laboratory Manual, by Sambrook et al. (eds.), 2nd ed., Cold Spring Harbor Laboratory Press, 1989; Lodie et al., Tissue Eng., 2002, 8(5):739-751); or as described in the Examples. Protein levels are determined using, Western blotting, ELISA,

enzymatic activity assays, or any other method for determining protein levels as described in, e.g., Current Protocols in Molecular Biology, by Ausubel et al. (eds.), John Wiley and Sons, 1998.

[0103] Expression levels may also be determined using reporter gene assays in cell/tissue extracts or by tissue or whole-animal imaging. In addition to MRI, tissue imaging on living animals can be performed by fluorescence detection (Hoffman Lancet Oncol., 2002 3:546-556; Tung et al., Cancer Res., 2000, 60:4953-4958), bioluminescence detection (Shi et al., PNAS, 2001, 98:12754-12759; Luke et al., J. Virol., 2002, 76:12149-12161; and U.S. Patent Nos. 5,650,135 and 6,217,847), positron emission tomography (Liang et al., Mol. Ther., 2002, 6:73-82, near-infrared fluorescence (Tung et al., Cancer Res., 2000, 60:4953-4958), or X-ray imaging (Hemminki et al., J. Nat. Cancer Inst., 2002, 94:741-749).

VIII. Neurological diseases

[0104] A neurological disease in methods 1-5 above can be a neurodegenerative disease such as, for example, ALS, Parkinson's disease, Alzheimer's disease, and Huntington's disease. The neurological disease can also be multiple sclerosis (MS), or other demyelinating diseases of the central or peripheral nervous system. Forms of MS in methods 1-5 can be: relapsing remitting MS (RRMS), secondary progressive MS (SPMS), primary progressive MS (PPMS), and malignant MS (Marburg Variant).

[0105] The subject being treated or administered the compound as per methods of the invention, is a mammal in need thereof, such as a subject in need of neuroprotection, including a subject who has or is at risk for developing a demyelinating and another specified neurodegenerative disease. The subject is mammalian, and can be a rodent or another laboratory animal, e.g., a non-human primate. In preferred embodiments, the subject is human.

[0106] Neurodegenerative diseases are described in, for example, Neurodegenerative Diseases: Neurobiology, Pathogenesis and Therapeutics, M. Flint Beal, Anthony E. Lang, Albert C. Ludolph, Cambridge University Press (July

11, 2005). Examples of neurological diseases suitable for the methods of the invention include neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, and Huntington's disease. Other examples include demyelinating neurological disease including, in addition to MS, the following diseases: acute haemorrhagic leucoencephalomyelitis, Hurst's disease, acute disseminated encephalomyelitis, optic neuritis, Devic's disease, spinal cord lesions, acute necrotizing myelitis, transverse myelitis, chronic progressive myelopathy, progressive multifocal leukoencephalopathy (PML), radiation myelopathy, HTLV-1 associated myelopathy, monophasic isolated demyelination, central pontine myelinolysis, and leucodystrophy (e.g., adrenoleucodystrophy, metachromatic leucodystrophy, Krabbe's disease, Canavan's disease, Alexander's disease, Pelizaeus-Merbacher disease, vanishing white matter disease, oculodentodigital syndrome, Zellweger's syndrome), chronic inflammatory demyelinating polyneuropathy (CIDP), acute inflammatory demyelinating polyneuropathy (AIDP), Leber's optic atrophy, and Charcot-Marie-Tooth disease.

[0107] Additional examples of diseases suitable for the methods of the invention include polyneuritis and mitochondrial disorders with demyelination. These disorders may be co-presented with, and possibly aggravated by diabetes, e.g., insulin-dependent diabetes mellitus (IDDM; type I diabetes), or other diseases.

IX. Animal models

[0108] A test compound may be further assayed in an animal model of MS, known as Experimental Autoimmune Encephalomyelitis (EAE) (Tuohy et al., J. Immunol., 1988, 141:1126-1130, Sobel et al. J. Immunol., 1984, 132:2393-2401, and Traugott, Cell Immunol., 1989 119:114-129). Chronic relapsing EAE provides a well established experimental model for testing agents that would be useful for the treatment of MS. The mouse EAE is an induced autoimmune demyelinating disease with many similarities to human MS in its clinical manifestations. In both EAE and MS, clinical disease is associated with

blood-brain barrier (BBB) dysfunction, infiltration of central nervous system by mononuclear cells (mainly macrophages and T lymphocytes, and serum products), and demyelination (Baker et al. J. Neuroimmunol., 1990, 28:261; Butter et al., J. Neurol. Sci., 1991, 104:9; Harris et al., Ann. Neurol., 1991, 29:548; Kermonde et al., Brain, 1990, 113:1477).

[0109] Clinical signs of MS and demyelinating pathology in EAE result from immunization with CNS myelin proteins or peptides (e.g., MBP, PLP, and MOG) under Th1 conditions (direct immunization model), or by adoptive transfer of CNS antigen-specific Th1 cells (adoptive transfer model) (Ben-Nun et al., Eur. J. Immunol., 1981, 11:195-199; Ando et al., Cell Immunol., 1989, 124:132-143; Zamvil et al., Nature, 1985, 317:355-358; Zamvil et al., Ann. Rev. Immunol., 1990, 8:579-621). For example, in the SJL mouse model of EAE, immunization with the CNS peptide PLP 139-151 or adoptive transfer of PLP-specific Th1 cells results in a disease course consisting of an acute phase with loss of tail tone on day 10 to day 12, followed by hind limb paralysis and CNS mononuclear cell infiltration (Tuohy et al., J. Immunol., 1988, 141:1126-1130, Sobel et al., J. Immunol., 1984, 132:2393-2401, and Traugott, Cell Immunol., 1989, 119:114-129). Resolution of clinical signs and recovery occurs on day 20 to day 25 and the animals may undergo several more relapses less severe than the initial phase. EAE has been used to evaluate new therapeutic approaches to T-cell-mediated autoimmune disease because of the clinical and histopathological similarities to the human demyelinating MS.

[0110] The ability of a compound to slow or prevent neurodegeneration (including demyelination and neuronal death) can be assessed in the EAE model or another animal model, including for example, Thieler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease, murine hepatitis virus (MHV), Semliki Forest Virus, or Sindbis virus as described in, e.g., Ercoli et al., J. immunol., 2006, 175:3293-3298.

[0111] A compound may be optionally tested in at least one additional animal model (see, generally, Immunologic Defects in Laboratory Animals, eds.

Gershwin et al., Plenum Press, 1981), for example, such as the following: the SWR X NZB (SNF1) mouse model (Uner et al., J. Autoimmune Disease, 1998, 11(3):233-240), the KRN transgenic mouse (K/BxN) model (Ji et al., Immunol. Rev., 1999, 69:139); NZB X NZW (B/W) mice, a model for SLE (Riemekasten et al., Arthritis Rheum., 2001, 44(10):2435-2445); the NOD mouse model of diabetes (Baxter et al., Autoimmunity, 1991, 9(1):61-67), etc.); or mouse models of multiple sclerosis (see, e.g., Linker et al., Eur. J. Immunol., 2002, 8(6):620-624, and Eugster et al., Nat. Med., 1999, 29:626-632; and Gold et al., Brain, 2006, 129:1953-1971).

X. Dosages and Formulations

[0112] Preliminary doses, for example, as determined in animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices. Toxicity and therapeutic efficacy can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD_{50}/ED_{50}. Compositions that exhibit large therapeutic indices are preferable.

[0113] The therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} (i.e., the concentration of the therapeutic compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture assays or animal models. Levels in plasma may be measured, for example, by ELISA or HPLC. The effects of any particular dosage can be monitored by a suitable bioassay. Examples of dosages are: about 0.1 x IC_{50}, about 0.5 x IC_{50}, about 1 x IC_{50}, about 5 x IC_{50}, 10 x IC_{50}, about 50 x IC_{50}, and about 100 x IC_{50}.

[0114] The data obtained from the in vitro assays or animal studies can be used in formulating a range of dosages for use in humans. Therapeutically

effective dosages achieved in one animal model can be converted for use in another animal, including humans, using conversion factors known in the art (see, e.g., Freireich et al., Cancer Chemother. Reports, 1966, 50(4):219-244 and Table 2 for Equivalent Surface Area Dosage Factors).

Table 2

To: / From:	Mouse (20 g)	Rat (150 g)	Monkey (3.5 kg)	Dog (8 kg)	Human (60 kg)
Mouse	1	1/2	1/4	1/6	1/12
Rat	2	1	1/2	1/4	1/7
Monkey	4	2	1	3/5	1/3
Dog	6	4	3/5	1	1/2
Human	12	7	3	2	1

[0115] The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. Generally, a therapeutically effective amount may vary with the subject's age, condition, and sex, as well as the severity of the medical condition in the subject. Examples of pharmaceutically acceptable dosages for compounds of the invention are from 1 µg/kg to 25 mg/kg, depending on the compounds, severity of the symptoms and the progression of the disease. The appropriate therapeutically effective doses can be selected by a treating clinician and would range approximately from 1 µg/kg to 20 mg/kg, from 1 µg/kg to 10 mg/kg, from 1 µg/kg to 1 mg/kg, from 10 µg/kg to 1 mg/kg, from 10 µg/kg to 100 µg/kg, from 100 µg to 1 mg/kg. Additionally, specific dosages indicated in the Examples or in the Physicians' Desk Reference (PDR).

[0116] For DMF or MMF, an effective amount can range from 1 mg/kg to 50 mg/kg (e.g., from 2.5 mg/kg to 20 mg/kg or from 2.5 mg/kg to 15 mg/kg). Effective doses will also vary, as recognized by those skilled in the art, dependent on route of administration, excipient usage, and the possibility of co-usage with other therapeutic treatments including use of other therapeutic agents. For example, an effective dose of DMF or MMR to be administered to a subject orally can be from about 0.1 g to 1 g per pay, 200 mg to about 800 mg per day (e.g., from about 240 mg to about 720 mg per day; or from about 480 mg to about 720 mg per day; or about 720 mg per day). For example, the 720 mg per day may be administered in separate administrations of 2, 3, 4, or 6 equal doses.

[0117] The dosage may be determined by a physician and adjusted, as necessary, to suit observed effects of the treatment. The compositions may be given as a bolus dose, to maximize the circulating levels for the greatest length of time after the dose. Continuous infusion may also be used after the bolus dose.

[0118] In some embodiments, compositions used in the methods of the invention further comprise a pharmaceutically acceptable excipient. As used herein, the phrase "pharmaceutically acceptable excipient" refers to any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. The compositions may also contain other active compounds providing supplemental, additional, or enhanced therapeutic functions. The pharmaceutical compositions may also be included in a container, pack, or dispenser together with instructions for administration.

[0119] A pharmaceutical composition is formulated to be compatible with its intended route of administration. Methods to accomplish the administration are known in the art. "Administration" is not limited to any particular delivery system and may include, without limitation, parenteral (including subcutaneous,

intravenous, intramedullary, intraarticular, intramuscular, or intraperitoneal injection), rectal, topical, transdermal, or oral (for example, in capsules (e.g., as, poweder, granules, microtablet, micropellets, etc.), suspensions, or tablets). Examples of some of formulations containing DMF and/or MMF are given in, e.g., US Patents Nos. 6,509,376, and 6,436,992.

[0120] Administration to an individual may occur in a single dose or in repeat administrations, and in any of a variety of physiologically acceptable salt forms, and/or with an acceptable pharmaceutical carrier and/or additive as part of a pharmaceutical composition. Physiologically acceptable salt forms and standard pharmaceutical formulation techniques and excipients are well known to persons skilled in the art.

[0121] The following Examples are intended for illustrative purposes and do not limit the invention as claimed.

EXAMPLES

Example 1: DMF and MMF are potent activators of Nrf2 at concentrations within clinical exposure range

[0122] Human colon carcinoma DLD1 cells were treated with DMF or MMF at indicated concentrations (5, 15, or 50 μM) for 16 hours, rinsed with PBS, and harvested into reducing SDS sample buffer. The lysates were subjected to SDS PAGE and the separated proteins were electrophoretically transferred onto nitrocellulose membranes for Western blot analysis. To detect Nrf2 and NQO1, the membranes were incubated with the respective primary antibodies overnight at 4ºC, washed, and incubated with peroxidase-conjugated secondary antibodies followed by the chemiluminescent peroxidase substrate. Detection of the target protein band luminescence and image acquisition were done using CCD-equipped imaging station Kodak2000R. The results shown in Figure 1, demonstrate that DMF and MMF are potent activators of Nrf2 at concentrations within clinical exposure range.

Example 2: DMF activates NQO1 in an Nrf2-dependent manner

[0123] DLD1 cells were grown in MEM supplemented with 10% fetal bovine serum. The cells were transfected with the indicated siRNA's using the Lipofectamine reagent (Invitrogen) according to the manufacturer's instructions and 30 hrs later stimulated with 30 μM DMF for 40 hours. The cells were harvested and processed for Western blotting analysis of Nrf2 and NQO1 levels as described in Example 1. Sources and the identity of reagents used in Examples 1 and 2 are specified Table 3 below:

	Target	Reagent	Source/Sequence	Vendor
Primary Antibody	Nrf2	Nrf2 (T-19)	goat polyclonal antibody	Santa Cruz Biotechnology
	Keap1	Keap1 (E-20)	goat polyclonal antibody	Santa Cruz Biotechnology
	NQO1	NQO1 (A180)	mouse monoclonal antibody	Santa Cruz Biotechnology
	GAPDH	Anti-GAPDH	mouse monoclonal antibody	Ambion
Secondary antibody	anti-mouse	HRP-Mouse IgG	sheep	Amersham Biosciences
	anti-rabbit	HRP-Rabbit IgG	donkey	Amersham Biosciences
	anti-Goat	HRP-Goat IgG	Bovine	Santa Cruz Biotechnology
siRNA	Nrf2	Nrf2-2	UCAUUGAACUGC UCUUUGGUU (antisense) (SEQ ID NO:17)	Dharmacon
	Keap1	Keap1-1	GAAUUAAGGCGG UUUGUCCUU (antisense) (SEQ ID NO:18)	Dharmacon

[0124] The results are shown in Figure 2 (for ease of representation, the image of the Western blot is turned upside down). The results demonstrate that DMF-induced upregulation of NQO1 requires Nrf2 and can be mimicked by

activation of Nrf2 through repression of Keap1. Therefore, DMF acts as an Nrf2 agonist causing cellular accumulation of Nrf2 and Nrf2 target gene expression.

* * *

[0125] All publications and patent documents cited herein are incorporated by reference in their entirety. To the extent the material incorporated by reference contradicts or is inconsistent with the present specification, the present specification will supersede any such material.

CLAIMS

1. A method of evaluating neuroprotective properties of a test compound, the method comprising:

a) contacting a cell with the test compound,

b) determining whether the Nrf2 pathway is upregulated in the cell,

c) determining whether the test compound slows or prevents demyelination, axonal loss, and/or neuronal death, and

d) selecting the test compound as a candidate for treating neurodegeneration in a neurological disease if 1) the Nrf2 pathway is upregulated and, optionally,

e) further determining whether demyelination, axonal loss, and/or neuronal death are/is prevented or slowed by the compound.

2. The method of claim 1, wherein the neurological disease is multiple sclerosis or another demyelinating disease.

3. The method of claim 1, wherein the cell in step a) is contacted with a plurality of test compounds.

4. The method of claim 1, wherein the Nrf2 pathway upregulation is determined by the levels of expression or activity of NQO1.

5. The method of claim 1, wherein the Nrf2 pathway is upregulated by at least 30% as indicated by one or more of the following parameters:

i) expression levels of endogenously produced or exogenously introduced Nrf2;

ii) subcellular localization and/or nuclear translocation of Nrf2;

iii) expression levels and/or activity of one or more genes under control of Nrf2 or an Nrf2-regulated reporter gene in an artificial reporter construct;

iv) levels of Nrf2 binding to the Nrf2-binding DNA element ARE;

v) stability of Nrf2/Keap1 complexes; and

vi) modification levels of Keap1 and other Nrf2/Keap1-associated proteins.

6. The method of claim 1, further comprising comparing the level of Nrf2 pathway upregulation by the test compound with a comparator compound.

7. The method of claim 6, wherein the comparator compound is dimethyl fumarate or monomethyl fumarate.

8. The method of claim 1, wherein the test compound has the structure of Formula I.

9. The method of claim 1, wherein the test compound is fumaric acid, its salt, or a fumaric acid derivative.

10. The method of claim 1, wherein the test compound is chosen from FTY 720, ABT-874, GSK683699, NeuroVax, MLN 1202, interferon γ, Tysabri™, Rituxan, TV 5010, NBI-788, MBP8298, Cladribine, Teriflunomide, Temsirolimus, and Laquinimod.

11. A method of treating a mammal having a neurological disease, comprising:

a) selecting a test compound according to the method of any one of claims 1-10, and

b) administering the selected test compound a mammal in need thereof, thereby treating neurodegeneration in the mammal.

12. A method of treating a mammal having a neurological disease by combination therapy, the method comprising:

a) administering to the mammal a therapeutically effective amount of a first compound that upregulates the Nrf2 pathway, and

b) administering a therapeutically effective amount of a second compound that does not upregulate the Nrf2 pathway.

13. The method of claim 12, wherein the first compound is fumaric acid, its salt, or a fumaric acid derivative.

14. The method of claim 11 or 12, wherein the neurological disease is multiple sclerosis or another demyelinating disease.

ABSTRACT OF THE DISCLOSURE

[0126] The disclosure provides methods of screening, identifying, and evaluating neuroprotective compounds useful for treatment of neurological diseases, such as, e.g., multiple sclerosis (MS). The compounds described upregulate the cellular cytoprotective pathway regulated by Nrf2. The disclosure further provides methods of utilizing such compounds in therapy for neurological disease, particularly, for slowing or reducing demyelination, axonal loss, or neuronal and oligodendrocyte death.

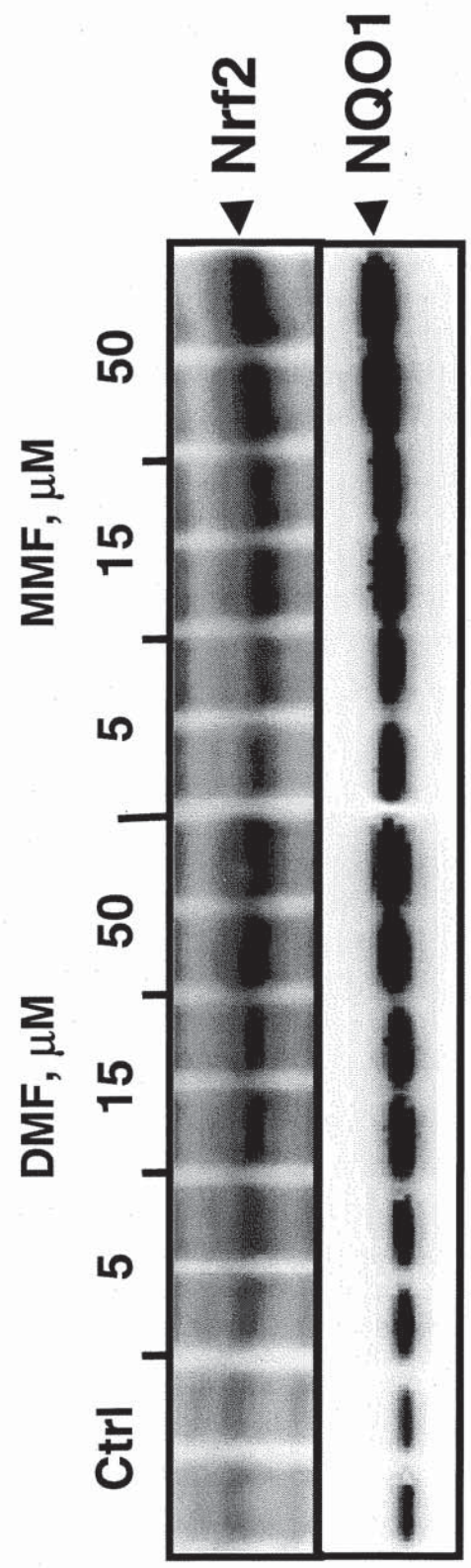


Fig. 1

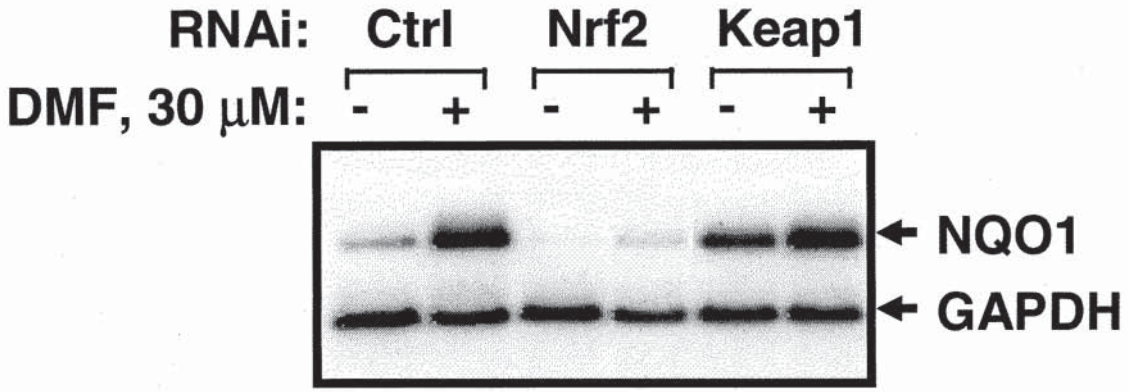


Fig. 2

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS
First Named Inventor/Applicant Name:	.. ..
Filer:	Konstantin Linnik/Kathleen Camire
Attorney Docket Number:	08201.6042-00000

Filed as Large Entity

Provisional Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Provisional application filing	1005	1	200	200
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	200

Electronic Acknowledgement Receipt	
EFS ID:	1502966
Application Number:	60888921
International Application Number:	
Confirmation Number:	1657
Title of Invention:	Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS
First Named Inventor/Applicant Name:	.. ..
Customer Number:	65779
Filer:	Konstantin Linnik/Kathleen Camire
Filer Authorized By:	Konstantin Linnik
Attorney Docket Number:	08201.6042-00000
Receipt Date:	08-FEB-2007
Filing Date:	
Time Stamp:	19:35:58
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment was successfully received in RAM	$200
RAM confirmation Number	983
Deposit Account	060916
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 and 1.17	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	TRANSMITTAL.pdf	78374	no	1
Warnings:					
Information:					
2	Specification	SPECIFICATION.pdf	2272724	no	36
Warnings:					
Information:					
3	Claims	CLAIMS.pdf	114425	no	3
Warnings:					
Information:					
4	Abstract	ABSTRACT.pdf	24940	no	1
Warnings:					
Information:					
5	Drawings	DRAWINGS.pdf	209266	no	2
Warnings:					
Information:					
6	Fee Worksheet (PTO-06)	fee-info.pdf	8145	no	2
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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
60/888,921	02/08/2007		200	08201.6042-00000	2		

CONFIRMATION NO. 1657

65779
BIOGEN IDEC / FINNEGAN HENDERSON, LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC20001-4413

FILING RECEIPT

Date Mailed: 02/21/2007

Receipt is acknowledged of this provisional Patent Application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please mail to the Commissioner for Patents P.O. Box 1450 Alexandria Va 22313-1450. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Power of Attorney: The patent practitioners associated with Customer Number 65779

If Required, Foreign Filing License Granted: 02/20/2007

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US60/888,921**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

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Title 37, Code of Federal Regulations, 5.11 & 5.15

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APPLICATION NUMBER	FILING OR 371 (c) DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NUMBER
60/888,921	02/08/2007		08201.6042-00000

CONFIRMATION NO. 1657

65779
BIOGEN IDEC / FINNEGAN HENDERSON, LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC 20001-4413

FORMALITIES LETTER

Date Mailed: 02/21/2007

NOTICE TO FILE MISSING PARTS OF PROVISIONAL APPLICATION

FILED UNDER 37 CFR 1.53(c)

Filing Date Granted

An application number and filing date have been accorded to this provisional application. The items indicated below, however, are missing. Applicant is given **TWO MONTHS** from the date of this Notice within which to file all required items and pay any fees required below to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- The provisional application cover sheet under 37 CFR 1.51(c)(1), which may be an application data sheet (37 CFR 1.76), is required identifying:
  - the name(s) of the inventor(s).
  - either city and state or city and foreign country of the residence of each inventor.

The applicant needs to satisfy supplemental fees problems indicated below.

The required item(s) identified below must be timely submitted to avoid abandonment:

- To avoid abandonment, a surcharge (for late submission of filing fee or cover sheet) as set forth in 37 CFR 1.16(g) of $50 for a non-small entity, must be submitted with the missing items identified in this letter.

SUMMARY OF FEES DUE:

Total additional fee(s) required for this application is **$50** for a non-small entity

- **$50** Surcharge.

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PART 3 - OFFICE COPY

PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION under 37 CFR 1.53(c).

Docket Number 08201.6042-00000		Type a plus sign (+) inside this box ≡	+

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
LUKASHEV	Matvey	E.	Tewksbury, MA

TITLE OF INVENTION (500 characters max)

Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS

CORRESPONDENCE ADDRESS

BIOGEN IDEC / FINNEGAN HENDERSON, LLP **Customer Number 65,779**

APPLICATION PARTS (previously submitted)

☒ Specification: 40 Pages ☒ Drawing(s): 2 Sheets/2 Figures

☐ Other: [Number] Pages; [Description]

METHOD OF PAYMENT

☐ A check or money order is enclosed to cover the Provisional Application filing fees ☒ The Commissioner is hereby authorized to charge any deficiency or credit any overpayment in fees to Deposit Account Number 06-0916.	PROVISIONAL FILING FEE - PAID $200.00 Total Number of Pages of specification, drawings, sequence or computer listing, or other papers 42. If more than 100 pages, add $250 for each additional 50 pages or fraction thereof. TOTAL FILING FEE $200.00 - PAID

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

[Text]

Respectfully submitted on behalf of the patent practitioners associated with Customer Number 65,779.

SIGNATURE _____ Date April 20, 2007

TYPED OR PRINTED NAME Konstantin M. Linnik REGISTRATION NO. 56,309

☐ Additional inventors are being named on separately numbered sheets attached hereto.

PROVISIONAL APPLICATION FILING ONLY

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	)	
	)	
Matvey E. LUKASHEV	)	
	)	
Application No.: 60/888,921	)	Confirmation No.: 1657
	)	
Filed: February 8, 2007	)	
	)	
For: Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS	)	

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Sir:

RESPONSE TO NOTICE TO FILE
MISSING PARTS OF PROVISIONAL APPLICATION

In response to the U. S. Patent Office's communication of February 21, 2007, Applicant submits a provisional application cover sheet and the required fee of $50.00.

The attached coversheet provides the name and residence of the inventor, as follows:

Matvey E. LUKASHEV

Tewksbury, MA.

Please issue an updated filing receipt for this application.

Please associate the enclosed cover sheet with the application, grant any extensions of time required to enter this response, and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: April 20, 2007

By: _____

Konstantin M. Linnik
Reg. No. 56,309
Tel.: (617) 452-1626

Electronic Patent Application Fee Transmittal

Application Number:	60888921
Filing Date:	08-Feb-2007
Title of Invention:	Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS
First Named Inventor/Applicant Name:	
Filer:	Konstantin Linnik/Kathleen Camire
Attorney Docket Number:	08201.6042-00000

Filed as Large Entity

Provisional Filing Fees

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Late provisional filing fee/cover sheet	1052	1	50	50
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Miscellaneous:				
			Total in USD ($)	50

Electronic Acknowledgement Receipt	
EFS ID:	1703231
Application Number:	60888921
International Application Number:	
Confirmation Number:	1657
Title of Invention:	Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS
First Named Inventor/Applicant Name:	
Customer Number:	65779
Filer:	Konstantin Linnik/Kathleen Camire
Filer Authorized By:	Konstantin Linnik
Attorney Docket Number:	08201.6042-00000
Receipt Date:	20-APR-2007
Filing Date:	08-FEB-2007
Time Stamp:	16:56:37
Application Type:	Provisional

Payment information:

Submitted with Payment	yes
Payment was successfully received in RAM	$50
RAM confirmation Number	699
Deposit Account	060916
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: Charge any Additional Fees required under 37 C.F.R. Section 1.16 and 1.17	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	Provisional_Cover_Sheet.pdf	126220	no	2
Warnings:					
Information:					
2	Fee Worksheet (PTO-06)	fee-info.pdf	8186	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	134406		

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New International Application Filed with the USPTO as a Receiving Office
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United States Patent and Trademark Office
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P.O. Box 1450
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www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
60/888,921	02/08/2007		250	08201.6042-00000		

CONFIRMATION NO. 1657

UPDATED FILING RECEIPT

65779
BIOGEN IDEC / FINNEGAN HENDERSON, LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC20001-4413

Date Mailed: 04/24/2007

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Applicant(s)

Matvey E. Lukashev, Tewsbury, MA;

Power of Attorney: The patent practitioners associated with Customer Number 65779

If Required, Foreign Filing License Granted: 02/20/2007

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US60/888,921**

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

Title

Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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to 37 CFR 5.15(b).

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	TOT CLAIMS	IND CLAIMS
60/888,921	02/08/2007		250	08201.6042-00000		

CONFIRMATION NO. 1657

65779
BIOGEN IDEC / FINNEGAN HENDERSON, LLP
901 NEW YORK AVENUE, NW
WASHINGTON, DC20001-4413

UPDATED FILING RECEIPT

Date Mailed: 04/25/2007

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Matvey E. Lukashev, Tewksbury, MA;

Power of Attorney: The patent practitioners associated with Customer Number 65779

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to 37 CFR 5.15(b).

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Provisional Patent Application of

UNKNOWN INVENTORS

Serial No.: 60/888,921

Filed: February 8, 2007

For: Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS

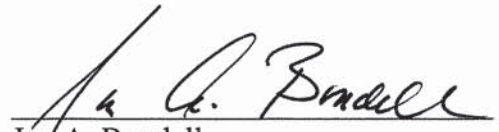
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR COPY OF APPLICATION

Sir:

Pursuant to 37 CFR 1.14(a)(1)(iv), the undersigned hereby requests a copy of the identified provisional patent application. A benefit of the provisional application has been claimed in an international patent application that was published in accordance with PCT Article 21(2) as WO 2008/097596. The Patent Office is authorized to charge our Deposit Account No. 120425 the appropriate fee under 37 CFR 1.19(b) in image format on compact disc or other physical electronic medium of $55.00.

Respectfully submitted,



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1040 Avenue of the Americas
New York, New York 10018
(212) 708-1800

Jay A. Bondell
Registration No. 28,188

Electronic Acknowledgement Receipt

EFS ID:	18330494
Application Number:	60888921
International Application Number:	
Confirmation Number:	1657
Title of Invention:	Nrf2 SCREENING ASSAYS AND RELATED METHODS AND COMPOSITIONS
First Named Inventor/Applicant Name:	Matvey E. Lukashev
Customer Number:	65779
Filer:	Jay A. Bondell
Filer Authorized By:	
Attorney Docket Number:	08201.6042-00000
Receipt Date:	28-FEB-2014
Filing Date:	08-FEB-2007
Time Stamp:	09:39:45
Application Type:	Provisional

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Miscellaneous Incoming Letter	Request_for_Copy_of_Application.pdf	33955 189b6603748ce8d164bc928d4414b7310df e698f	no	1

Warnings:	
Information:	

Total Files Size (in bytes):	33955

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