

(43) International Publication Date
15 September 2016 (15.09.2016)

(51) International Patent Classification:

A61K 31/52 (2006.01) C07D 413/04 (2006.01)
A61P 25/02 (2006.01)

(21) International Application Number:

PCT/US2016/021813

(22) International Filing Date:

10 March 2016 (10.03.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/131,053 10 March 2015 (10.03.2015) US

(71) Applicant: UNIVERSITY OF FLORIDA RESEARCH
FOUNDATION, INC. [US/US]; 223 Grinter Hall,
Gainesville, FL 32611 (US).

(72) Inventor: NOTTERPEK, Lucia; 5514 NW 48th Place,
Gainesville, FL 32606 (US).

(74) Agent: WEATHERHEAD, Robin, A.; Wolf, Greenfield
& Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02110-
2206 (US).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: TREATMENT OF PERIPHERAL NEUROPATHIES

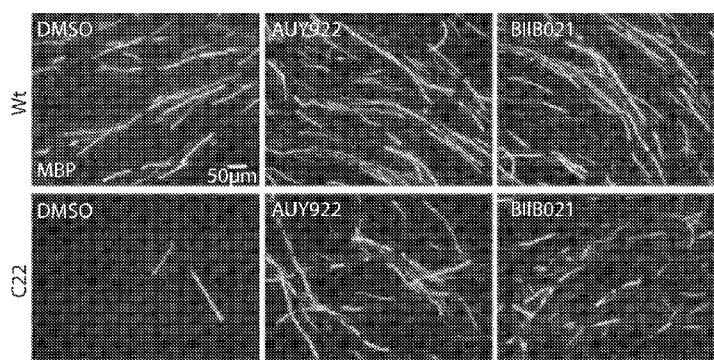


Figure 3E

(57) Abstract: The present disclosure relates to methods and compositions to treat peripheral neuropathies, including demyelinating neuropathies and hereditary neuropathies, *e.g.*, Charcot-Marie-Tooth disease. The invention provides methods and compositions comprising heat shock protein 90 (HSP90) inhibitors for the treatment of neuropathies.

TREATMENT OF PERIPHERAL NEUROPATHIES

RELATED APPLICATIONS

[0001] The present application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application, U.S.S.N. 62/131,053, filed March 10, 2015, which is incorporated herein by reference.

GOVERNMENT SUPPORT

[0002] This invention was made with U.S. government support under grant numbers NS091435 and NS041012, awarded by the National Institutes of Health. The U.S. government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] Peripheral demyelinating neuropathies are a group of peripheral nervous system disorders, including hereditary neuropathies. Symptoms of demyelinating neuropathies include sensory symptoms, such as numbness, tingling, and pain in the extremities; motor symptoms, such as weakness and loss of muscle tissue, as well as physical abnormalities, such as high arches, hammer toes, thin calf muscles, and scoliosis. There is no standard treatment or cure for demyelinating neuropathies. Most treatments are symptomatic, and include physical therapy, pain management, orthopedic braces, and orthopedic surgery. Accordingly, there is a need for novel compositions and methods to treat demyelinating neuropathies.

SUMMARY OF THE INVENTION

[0004] Provided herein are methods for treating neuropathies by administering a compound that interacts with heat shock protein 90 (HSP90) to a subject in need thereof. The compound may be an inhibitor of HSP90, a modulator of HSP90, a binder of HSP90, or a compound that modifies HSP90. In certain embodiments, the method comprises administering an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof, to a subject in need thereof. In some embodiments, the method comprises administering an HSP90 inhibitor, or a pharmaceutically acceptable salt thereof. In some embodiments, the method comprises administering a pharmaceutical composition comprising an HSP90 inhibitor. In certain embodiments, the method comprises administering BIIB021, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition

thereof. In some embodiments, the method comprises administering BIIB021, or a pharmaceutically acceptable salt thereof. In some embodiments, the method comprises administering a pharmaceutical composition comprising BIIB021. In certain embodiments, the method comprises administering the HSP90 inhibitor AUY922, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the method comprises administering AUY922 or a pharmaceutically acceptable salt thereof. In some embodiments, the method comprises administering a pharmaceutical composition comprising AUY922.

[0005] In another aspect, provided herein are pharmaceutical compositions for the treatment of a neuropathy. The pharmaceutical composition may comprise a compound that interacts with HSP90. The compound may be an inhibitor of HSP90 (*e.g.*, BIIB021, AUY922), modulator of HSP90, binders of HSP90, or a compound that modifies HSP90. In certain embodiments, the compound is an HSP90 inhibitor, or pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, or prodrug thereof, and optionally a pharmaceutically acceptable excipient. In certain embodiments, the pharmaceutical composition comprises BIIB021, or pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises AUY922, or pharmaceutically acceptable salt thereof.

[0006] In an additional aspect, provided herein are kits for the treatment of a neuropathy. The kit may comprise an HSP90 inhibitor, or a pharmaceutically acceptable salt or composition thereof, and a container. In certain embodiments, the kit includes a first container comprising an HSP90 inhibitor, or a pharmaceutically acceptable salt or composition thereof; and instructions for administering the HSP90 inhibitor, or a pharmaceutically acceptable salt or composition thereof, to the subject to treat and/or prevent the pathological condition. In some embodiments, the HSP90 inhibitor is BIIB021, or pharmaceutically acceptable salt thereof. In some embodiments, the HSP90 inhibitor is AUY922, or pharmaceutically acceptable salt thereof.

[0007] The methods, compositions, kits, and compounds described herein may be used for the treatment of neuropathies. In certain embodiments, the neuropathy is a peripheral demyelinating neuropathy. In certain embodiments, the neuropathy is a hereditary neuropathy. In certain embodiments, the neuropathy is caused by diabetes, impaired glucose tolerance, or Lyme disease. In certain embodiments, the neuropathy is a chemotherapy-induced peripheral neuropathy (CIPN). In certain embodiments, the neuropathy is anti-MAG peripheral neuropathy, chronic inflammatory demyelinating polyneuropathy (CIDP),

Guillain-Barré syndrome, hereditary neuropathy with liability to pressure palsy (HNPP), or progressive inflammatory neuropathy. In certain embodiments, the neuropathy is Charcot-Marie-Tooth disease (CMT), Dejerine-Sottas disease, congenital hypomyelinating neuropathy (CHN), Russe-type hereditary motor and sensor neuropathy (HMSNR), CMT with pyramidal features, CMT with optic atrophy, Cowchock syndrome, Rosenberg-Chutorian syndrome, or Roussy-Lévy syndrome. In certain embodiments, the neuropathy is Charcot-Marie-Tooth disease type 1A (CMT1A). In certain embodiments, the neuropathy is associated with defective myelin in the myelin sheath of nerve cells. In certain embodiments, the neuropathy is associated with axonal shrinkage and atrophy. In certain embodiments, the neuropathy is associated with overexpression of PMP22. In certain embodiments, the neuropathy is associated with misfolding or aggregation of peripheral myelin protein 22 (PMP22). In some embodiments, the neuropathy is associated with an accumulation of cytosolic aggregates of peripheral myelin protein 22 (PMP22). In some embodiments, the neuropathy is ameliorated by activation of the heat shock protein response.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] The accompanying drawings, which constitute a part of this specification, illustrate several embodiments of the invention and together with the description, serve to explain the principles of the invention.

[0009] FIGURES 1A-1B. Effects of HSP90 inhibitors on Schwann cells. *Figure 1A:* Cell viability after treatment with DMSO, Geldanamycin (GA) (50 nM) or the indicated five HSP90 inhibitors (50 and 500 nM) for 24 hours was calculated as percentage of DMSO (vehicle) and graphed. *Figure 1B:* HSP70 mRNA levels were quantified after 24 hours treatment with the indicated compounds (100 nM). GAPDH was used as an internal control. (A, B) GA served as a positive control. Graphs plotted as means + S.E.M (n=3 independent experiments); *** $P<0.001$; ** $P<0.01$; * $P<0.05$; n.s. non-significant; two-tailed Unpaired Student's *t*-test.

[0010] FIGURES 2A-2C. Treatment with BIIB021 and AUY922 increase chaperone expression in a dose- and time-dependent manner. *Figure 2A:* Steady-state levels of HSP70 and HSP27 in whole Schwann cell lysates (15 µg/lane; n=3 independent experiments) were analyzed after 24 hours treatment with DMSO, BIIB021 or AUY922, at the specified doses. GA (50 nM) served as a positive control. *Figure 2B:* HSP70 and HSP27 levels (15 µg/lane; n=3 independent experiments) were observed after treatment with 100 nM of BIIB021 or AUY922 for the indicated times. *Figure 2C:* Chaperone pathway activation by BIIB021 or

AUY922 (100 nM) was studied after 2 hours or 4 hours (treatment) at 4, 24, 32 and 48 hours chase time points (15 µg/lane; n=3 independent experiments). (A, B) GAPDH and (C) tubulin served as loading controls. Molecular mass on left, in kDa.

[0011] FIGURES 3A-3E. Improved myelin production in dorsal-root ganglion (DRG) explant cultures from C22 mice after treatment with AUY922 and BIIB021. *Figure 3A:* Steady-state levels of HSP70 and P0 were analyzed in DMSO (Ct), AUY922 (A) or BIIB021 (B) explant lysates (35 µg/ lane; n=4 independent experiments). Tubulin serves as a protein loading control. Molecular mass on left, in kDa. *Figure 3B:* Wild type (Wt) DRG cultures, with Schwann cells (Neuron+Schwann cell) and without Schwann cells (Schwann cell depleted) were treated with 100 nM AUY922 and analyzed for the indicated chaperones (35 µg/lane; n=3 independent experiments). Tubulin serves as a protein loading control. Molecular mass on left, in kDa. *Figures 3C and 3D:* Myelin basic protein (MBP) positive myelin internode lengths in explant cultures from Wild type (Wt) (*Figure 3C*) and C22 (*Figure 3D*) embryos treated with DMSO, AUY922 or BIIB021 were measured (n=100-120 segments per group). Graphs plotted as means + S.E.M.; *** $P<0.001$; * $P<0.05$; n.s. non-significant; two-tailed Unpaired Student's *t*-test. *Figure 3E:* Cultures from Wild type (Wt) (top panel) and C22 (lower panel) embryos, treated with the indicated compounds were stained for MBP (green) (n= 4 independent experiments). Nuclei were visualized with Hoechst dye (blue). Scale bar, as shown.

[0012] FIGURES 4A-4D. Treatment with AUY922 improves neuromuscular performance of C22 mice. *Figure 4A:* Body weights of Wild type (Wt) and C22 mice (n=6-8 mice per group) were plotted over the treatment period. *Figures 4B-4C:* Performance of animals on the accelerating rotarod at (*Figure 4B*) baseline (7 weeks age) and (*Figure 4C*) at the end of the treatment (25 weeks age) are shown. Two-tailed Unpaired Student's *t*-test; graphs plotted as means + S.E.M.; *** $P<0.001$; ** $P<0.01$; * $P<0.05$; n.s. non-significant. *Figure 4D:* Muscle force, analyzed using an *in situ* technique, was recorded and normalized to the animal's body weight (mN/g=milliNewtons/grams). Two-way ANOVA with Sidak's multiple comparison between individual groups and frequencies; graph plotted as means + S.E.M.; *** $P<0.001$; ** $P<0.01$; * $P<0.05$; n.s. non-significant.

[0013] FIGURES 5A-5D. Bioactivity of AUY922 in liver and sciatic nerves. *Figure 5A:* Whole liver lysates (30 µg/lane) from vehicle (Veh) and AUY922 (AUY)- treated animals were assessed for levels of HSP70 and HSP27. GAPDH and tubulin serve as loading controls. Molecular mass on left, in kDa. *Figure 5B:* Steady-state levels of the same chaperones were studied in the sciatic nerves (30 µg/lane). GAPDH and tubulin serve as

loading controls. Molecular mass on left, in kDa. *Figure 5C*: Sciatic nerve lysates (5 µg/lane) were treated with either EndoH (H) or PNGaseF (N) and probed with anti-human PMP22 antibodies. No enzyme samples served as control (C). Arrows point towards the EndoH-resistant PMP22 fractions while the arrowheads indicate to the EndoH-sensitive fractions. *Figure 5D*: Graph showing densitometric quantification of EndoH-resistant PMP22 fractions in sciatic nerves (n=6-8 mice per group), plotted as means + S.E.M.; *** $P < 0.001$; * $P < 0.05$; n.s. non-significant; two-tailed Unpaired Student's *t*-test.

[0014] FIGURES 6A-6H. AUY922 administration supports the maintenance of myelinated axons in sciatic nerves of C22 mice. *Figure 6A*: Cross-sectional views of nerve sections from Wild type (Wt) (top panels) and C22 (lower panels) male mice. Micron bar, 45 µm. *Figure 6B*: Cross-sectional area occupied by nerve fibers in a 40 µm×40 µm square (n=20-25 fibers per animal; n=6-8 mice per group) was measured and graphed as shown. Graph plotted as means + S.E.M.; *** $P < 0.001$, across the treatment groups; # $P < 0.05$, across the genotypes; two-tailed Unpaired Student's *t*-test. *Figures 6C-6D*: Correlative analysis between axon and fiber diameter measurements (n=950-1100 fibers per group) were obtained from cross-sectional areas of the sciatic nerves in (*Figure 6C*) Wild type (Wt) and (*Figure 6D*) C22 groups. *Figure 6E*: Comparison of trend lines between the cohorts in C and D. *Figures 6F-6G*: Scatter plots comparing the g-ratios (axon diameter/fiber diameter) of individual fibers plotted as a function of axon diameters in nerves of (*Figure 6F*) Wild type (Wt) and (*Figure 6G*) C22 animals (n=950-1100 fibers per group). *Figure 6H*: Trend lines comparisons of graphs in F and G.

[0015] FIGURES 7A-7B. AUY922 reduces the frequency of PMP22 aggregates in sciatic nerves of C22 mice. *Figure 7A*: PMP22-positive aggregates per microscopic field (0.1 mm²) were counted in longitudinal sections of sciatic nerves (n=6-8 mice per group) stained with anti-human and anti-rat PMP22 antibodies. Graph plotted as means + S.E.M.; * $P < 0.05$; n.s. non-significant; two-tailed Unpaired Student's *t*-test. *Figure 7B*:

Representative images of PMP22 (red) stained nerve sections from Wild type (Wt) (insets) and C22 male mice are shown. Arrows point towards PMP22-positive aggregates. Hoechst dye (blue) was used to visualize the nuclei. Scale bars, as shown.

DEFINITIONS

[0016] As used herein, the term “neuropathy” refers to a disease, disorder, or condition associated with damage and/or inflammation in nerves. Neuropathies are a subset of neurological diseases. The damage may be a symptom of another disease (*e.g.*, diabetes,

impaired glucose tolerance, Lyme disease), may be caused by injury or other external factors (*e.g.*, infection, medication, radiation, chemotherapy), or the damage may be the pathology of the disease itself, such as in the case of hereditary neuropathies or idiopathic neuropathies. Neuropathies can affect both the central and peripheral nervous system, and may affect a single nerve, multiple nerves, or may be a polyneuropathy. Neuropathies can be chronic or acute and can affect any type of nerve or multiple types (*e.g.*, motor, sensory, autonomic). Polyneuropathies are characterized by damage to many nerve cells often in various parts of the body. Polyneuropathies may be classified based on the part of the nerve cell most affected by the condition, including the axon, myelin sheath, or cell body.

[0017] The term “demyelinating”, as used herein, refers to defects in the myelin sheath of nerve cells. This includes defective myelin in the myelin sheath of nerve cells, or loss of or damage to Schwann cells. Schwann cells are glial cells of the peripheral nervous system which synthesize myelin and the myelin sheath. Demyelination is associated with slow or blocked conduction of action potentials in nerve cell axons, which can lead to neuropathic symptoms. Demyelinating neuropathies or myelinopathies are neuropathies in which the primary defects occur in the myelin sheath. Axon-origin neuropathies or axonopathies are neuropathies associated with degeneration of axons in the nerve cells of the peripheral nervous system.

[0018] Examples of neuropathies of the peripheral nervous system include but are not limited to: anti-MAG peripheral neuropathy, Charcot-Marie-Tooth disease (CMT) (*e.g.*, CMT1A, Dejerine-Sottas disease, congenital hypomyelinating neuropathy (CHN), Russe-type hereditary motor and sensor neuropathy (HMSNR), CMT with pyramidal features, CMT with optic atrophy, Cowchock syndrome, Rosenberg-Chutorian syndrome, Roussy-Lévy syndrome), chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain-Barré syndrome, hereditary neuropathy with liability to pressure palsy (HNPP), progressive inflammatory neuropathy, and chemotherapy-induced peripheral neuropathy (CIPN).

[0019] Charcot-Marie-Tooth disease (CMT) refers to a group of genetic disorders of the peripheral nervous system that may be caused by mutations in a number of genes. CMT symptoms include balance difficulties, clumsiness, muscle weakness in the foot, legs, and/or hands, foot abnormalities (*e.g.*, high arches, flat feet, hammer toes), leg and calf muscle abnormalities, difficulty flexing the foot, decreased sensitivity in the extremities, loss of hearing, and loss of vision. CMT can be classified according to type or subtype. Types of CMT include, but are not limited to, CMT1, CMT2, CMT3, CMT4, CMT5, CMT6, CMTDI, CMTRI, and CMTX. CMT1 is classified into subtypes, which include, but are not limited to,

CMT1A, CMT1B, CMT1C, CMT1D, CMT1E, and CMT1F. CMT1A is the most common form of Charcot-Marie-Tooth disease, and is associated with a mutation of the *PMP22* gene. Other subtypes associated with a mutation of the *PMP22* gene include CMT1E and CMT3. The mutation causing Charcot-Marie-Tooth disease may not always be known, and methods and compositions herein are not limited to treatment of types associated with a *PMP22* mutation. Certain types CMT are sometimes referred to by other disease names, including: Dejerine-Sottas disease (CMT3), congenital hypomyelinating neuropathy (CMT4E), Russe-type hereditary motor and sensor neuropathy (CMT4G), CMT with pyramidal features (CMT5), CMT with optic atrophy (CMT6), Cowchock syndrome (CMTX4), Rosenberg-Chutorian syndrome (CMTX5), and Roussy- Lévy syndrome (associated with both the CMT1A and CMT2A phenotype).

[0020] Neuropathies may be caused by an underlying disorder or certain external factors. Examples of diseases or conditions that may cause a neuropathy include, diabetes, impaired glucose tolerance, renal failure, connective tissue diseases, malnutrition, and alcoholism. Examples of external factors that may cause a neuropathy include exposure to toxin or drugs, *e.g.*, chemotherapy drugs.

[0021] The term “peripheral myelin protein 22” or “PMP22” refers to a protein that is encoded by the *PMP22* gene. PMP22 protein is found in the peripheral nervous system as a component of myelin. PMP22 is produced in Schwann cells and is incorporated in myelin as a component of the myelin sheath. PMP22 is prone to misfolding and aggregation, which may lead to myelin defects as a result of insufficient properly folded PMP22 protein being delivered to the plasma membrane of Schwann cells. Non-limiting examples of the nucleotide and protein sequences for human PMP22 are described in GenBank Accession Numbers NC_000017.11 (nucleotide) and CAG46729.1 (protein), incorporated herein by reference.

The amino acid sequence of this human PMP22 is as follows:

```
MLLLLLSIIVLHVAVLVLLFVSTIVSQWIVGNHATDLWQNCSTSSSGNVHHCFS SSPNEWLQSVQATMILS
IIFSILSLFLFFCQLFTLTGGRFYITGIFQILAGLCVMSAAAIYTVRHPEWHLNSDYSYGFAYILAWVAFP
LALLSGVIYVILRKRE (SEQ ID NO:1).
```

[0022] The term “heat shock protein” or “HSP” refers to a family of proteins that may be upregulated by cells in response to stress, including heat shock (HS). Many heat shock proteins are chaperone proteins that interact with other proteins to promote correct folding, repair misfolded proteins, prevent protein aggregation, and/or promote degradation or deaggregation of protein aggregates. Heat shock proteins may play additional roles, such as intracellular transport and cell signaling. Exemplary eukaryotic HSPs include, HSP10,

HSP27, HSPB6, HSPB1, HSP40, HSP60, HSP71, HSP70, HSP72, GRP78, HSP90, GRP94, HSP104, and HSP110.

[0023] The heat shock proteins include “heat shock protein 90” or “HSP90”, which is a chaperone protein with many cellular roles, including assisting protein folding, intracellular transport, protein maintenance, protein degradation, and cell signaling. Some isoforms of HSP90 comprise part of a chaperone complex with heat shock factor 1 (HSF1), effectively inactivating HSF1 by preventing translocation to the nucleus and interaction of HSF1 with the heat shock elements which initiate heat shock gene transcription. Cytosolic isoforms of HSP90 in humans include HSP90- α 1, HSP90- α 2, and HSP90- β , which are encoded by genes *HSP90AA1*, *HSP90AA2*, and *HSP90AB1*, respectively. The HSP90 inhibitor of the present invention may be an inhibitor of one or more, or all isoforms of HSP90. Non-limiting examples of the nucleotide and protein sequences for human HSP90- α 1 (*HSP90AA1*) are described in GenBank Accession Numbers NC_000014.9 (nucleotide) and NP_001017963.2 (protein), incorporated herein by reference. The amino acid sequence of this human HSP90- α 1 is as follows:

```
MPPCSGGDGSTPPGPSLRDRDCPAQSAEYPRDRLDPRPGSPSEASSPPFLRSRAPVNWYQEKAQVFLWHLMV
SGSTTLCLWKQPFHVSAFPVTASLAFRQSQGAGQHLYKDLQPFILLRLLMPEETQTQDQPMEEEEVETFAF
QAEIAQLMSLIINTFYSNKEIFLRELISNSSDALDKIRYESLTDPSKLD SGKELHINLIPNKQDRTLTIIVDT
GIGMTKADLINNLGTIAKSGTKAFMEALQAGADISMIGQFVGVFYSAYLVAEKVTVITKHNDDEQYAWESSA
GGSFTVRTDTGEPMGRGTKVILHLKEDQTEYLEERRIKEIVKKHSQFIGYPITL FVEKERDKEVSDDEAEK
EDKEEKEKEKEKESEDKPEIEDVGSDEEEEEKKDGDKKKKKKIKEKYIDQEELNKT KPIWTRNPDDITNEEYG
EFYKSLTNDWEDHLAVKHFSVEGQLEFRALLFVPRRAPFDLFENRKKKNNIKLYVRRVFIMDNCEELIPEYL
NFIRGVVDS EDLPLNISREMLQQSKILKVIKRNLVKKCLELFTELAEDKENYKKFYEQFSKNIKLGIHEDSQ
NRKKLSELLRYYTSASGDEMVS LKDYCTRMKENQKHIYYITGETKDQVANS AFVERLRKHGLEVIYMI EPID
EYCVQQLKEFEGKTLVSVTKEGLELPEDEEEKKKQEEKTKFENLCKIMKDILEKKVEKVVVSNRLVTSPCC
IVTSTYGTWIANMERIMKAQALRDNSTMGYMAAKKHLEINPDHSIIETLRQKAEADKNDKSVKDLVILLYETA
LLSSGFSLEDPQTHANRIYRMIKLG LGIDEDDPTADDTSAAVTEEMP PLEGGDDDTSRMEEVD (SEQ ID
NO:2) .
```

[0024] The term “pharmaceutically acceptable salt” refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1–19, incorporated herein by reference. Pharmaceutically acceptable salts of the compounds of this invention include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically

acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids, such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid or by using other methods known in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium, and $N^+(C_{1-4} \text{ alkyl})_4^-$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate.

[0025] The term “solvate” refers to forms of the compound, or a salt thereof, that are associated with a solvent, usually by a solvolysis reaction. This physical association may include hydrogen bonding. Conventional solvents include water, methanol, ethanol, acetic acid, DMSO, THF, diethyl ether, and the like. The compounds described herein may be prepared, *e.g.*, in crystalline form, and may be solvated. Suitable solvates include pharmaceutically acceptable solvates and further include both stoichiometric solvates and non-stoichiometric solvates. In certain instances, the solvate will be capable of isolation, for example, when one or more solvent molecules are incorporated in the crystal lattice of a crystalline solid. “Solvate” encompasses both solution-phase and isolatable solvates. Representative solvates include hydrates, ethanolates, and methanolates.

[0026] The term “hydrate” refers to a compound that is associated with water. Typically, the number of the water molecules contained in a hydrate of a compound is in a definite ratio to the number of the compound molecules in the hydrate. Therefore, a hydrate of a compound may be represented, for example, by the general formula $R \cdot x H_2O$, wherein R

is the compound, and x is a number greater than 0. A given compound may form more than one type of hydrate, including, *e.g.*, monohydrates (x is 1), lower hydrates (x is a number greater than 0 and smaller than 1, *e.g.*, hemihydrates ($R \cdot 0.5 H_2O$)), and polyhydrates (x is a number greater than 1, *e.g.*, dihydrates ($R \cdot 2 H_2O$) and hexahydrates ($R \cdot 6 H_2O$)).

[0027] The term “tautomers” or “tautomeric” refers to two or more interconvertible compounds resulting from at least one formal migration of a hydrogen atom and at least one change in valency (*e.g.*, a single bond to a double bond, a triple bond to a single bond, or *vice versa*). The exact ratio of the tautomers depends on several factors, including temperature, solvent, and pH. Tautomerizations (*i.e.*, the reaction providing a tautomeric pair) may catalyzed by acid or base. Exemplary tautomerizations include keto-to-enol, amide-to-imide, lactam-to-lactim, enamine-to-imine, and enamine-to-(a different enamine) tautomerizations.

[0028] It is also to be understood that compounds that have the same molecular formula but differ in the nature or sequence of bonding of their atoms or the arrangement of their atoms in space are termed “isomers”. Isomers that differ in the arrangement of their atoms in space are termed “stereoisomers”.

[0029] Stereoisomers that are not mirror images of one another are termed “diastereomers” and those that are non-superimposable mirror images of each other are termed “enantiomers”. When a compound has an asymmetric center, for example, it is bonded to four different groups, a pair of enantiomers is possible. An enantiomer can be characterized by the absolute configuration of its asymmetric center and is described by the R- and S-sequencing rules of Cahn and Prelog, or by the manner in which the molecule rotates the plane of polarized light and designated as dextrorotatory or levorotatory (*i.e.*, as (+) or (-)-isomers respectively). A chiral compound can exist as either individual enantiomer or as a mixture thereof. A mixture containing equal proportions of the enantiomers is called a “racemic mixture”.

[0030] The term “polymorph” refers to a crystalline form of a compound (or a salt, hydrate, or solvate thereof). All polymorphs have the same elemental composition. Different crystalline forms usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and solubility. Recrystallization solvent, rate of crystallization, storage temperature, and other factors may cause one crystal form to dominate. Various polymorphs of a compound can be prepared by crystallization under different conditions.

[0031] The term “prodrugs” refers to compounds that have cleavable groups and become by solvolysis or under physiological conditions the compounds described herein, which are pharmaceutically active *in vivo*. Such examples include, but are not limited to, choline ester derivatives and the like, N-alkylmorpholine esters and the like. Other derivatives of the compounds described herein have activity in both their acid and acid derivative forms, but in the acid sensitive form often offer advantages of solubility, tissue compatibility, or delayed release in the mammalian organism (see, Bundgard, H., *Design of Prodrugs*, pp. 7-9, 21-24, Elsevier, Amsterdam 1985). Prodrugs include acid derivatives well known to practitioners of the art, such as, for example, esters prepared by reaction of the parent acid with a suitable alcohol, or amides prepared by reaction of the parent acid compound with a substituted or unsubstituted amine, or acid anhydrides, or mixed anhydrides. Simple aliphatic or aromatic esters, amides, and anhydrides derived from acidic groups pendant on the compounds described herein are particular prodrugs. In some cases it is desirable to prepare double ester type prodrugs such as (acyloxy)alkyl esters or ((alkoxycarbonyl)oxy)alkylesters. C₁-C₈ alkyl, C₂-C₈ alkenyl, C₂-C₈ alkynyl, aryl, C₇-C₁₂ substituted aryl, and C₇-C₁₂ arylalkyl esters of the compounds described herein may be preferred.

[0032] The terms “composition” and “formulation” are used interchangeably.

[0033] A “subject” to which administration is contemplated refers to a human (*i.e.*, male or female of any age group, *e.g.*, pediatric subject (*e.g.*, infant, child, or adolescent) or adult subject (*e.g.*, young adult, middle-aged adult, or senior adult)) or non-human animal. In certain embodiments, the non-human animal is a mammal (*e.g.*, primate (*e.g.*, cynomolgus monkey or rhesus monkey), commercially relevant mammal (*e.g.*, cattle, pig, horse, sheep, goat, cat, or dog), or bird (*e.g.*, commercially relevant bird, such as chicken, duck, goose, or turkey)). In certain embodiments, the non-human animal is a fish, reptile, or amphibian. The non-human animal may be a male or female at any stage of development. The non-human animal may be a transgenic animal or genetically engineered animal. The terms “administer,” “administering,” or “administration,” as used herein, refer to implanting, absorbing, ingesting, injecting, inhaling, or otherwise introducing an inventive compound, or a pharmaceutical composition thereof, in or on a subject.

[0034] The term “administer,” “administering,” or “administration” refers to implanting, absorbing, ingesting, injecting, inhaling, or otherwise introducing a compound described herein, or a composition thereof, in or on a subject.

[0035] The terms “treatment,” “treat,” and “treating” refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease described herein. In some embodiments, treatment may be administered after one or more signs or symptoms of the disease have developed or have been observed. In other embodiments, treatment may be administered in the absence of signs or symptoms of the disease. For example, treatment may be administered to a susceptible subject prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of exposure to a pathogen). Treatment may also be continued after symptoms have resolved, for example, to delay or prevent recurrence.

[0036] As used herein, the terms “condition,” “disease,” and “disorder” are used interchangeably. The terms “genetic” and “hereditary” are also used interchangeably herein.

[0037] An “effective amount” of a compound described herein refers to an amount sufficient to elicit the desired biological response. An effective amount of a compound described herein may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the compound, the condition being treated, the mode of administration, and the age and health of the subject. In certain embodiments, an effective amount is a therapeutically effective amount. In certain embodiments, an effective amount is a prophylactic treatment. In certain embodiments, an effective amount is the amount of a compound described herein in a single dose. In certain embodiments, an effective amount is the combined amounts of a compound described herein in multiple doses.

[0038] A “therapeutically effective amount” of a compound described herein is an amount sufficient to provide a therapeutic benefit in the treatment of a condition or to delay or minimize one or more symptoms associated with the condition. A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the condition. The term “therapeutically effective amount” can encompass an amount that improves overall therapy, reduces or avoids symptoms, signs, or causes of the condition, and/or enhances the therapeutic efficacy of another therapeutic agent. In certain embodiments, a therapeutically effective amount is an amount sufficient for repairing or improving the formation of myelin in the myelin sheath of nerve cells. In certain embodiments, a therapeutically effective amount is an amount sufficient for treating a peripheral neuropathy. In certain embodiments, a therapeutically effective amount is an amount sufficient for repairing or improving the formation of myelin in the myelin sheath of nerve cells and treating a peripheral neuropathy.

[0039] A “prophylactically effective amount” of a compound described herein is an amount sufficient to prevent a condition, or one or more symptoms associated with the

condition or prevent its recurrence. A prophylactically effective amount of a compound means an amount of a therapeutic agent, alone or in combination with other agents, which provides a prophylactic benefit in the prevention of the condition. The term “prophylactically effective amount” can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of another prophylactic agent. In certain embodiments, a prophylactically effective amount is an amount sufficient for repairing or improving the formation of myelin in the myelin sheath of nerve cells. In certain embodiments, a prophylactically effective amount is an amount sufficient for treating a peripheral neuropathy. In certain embodiments, a prophylactically effective amount is an amount sufficient for repairing or improving the formation of myelin in the myelin sheath of nerve cells and treating a peripheral neuropathy.

[0040] The term “biological sample” refers to any sample including tissue samples (such as tissue sections and needle biopsies of a tissue); cell samples (*e.g.*, cytological smears (such as Pap or blood smears) or samples of cells obtained by microdissection); samples of whole organisms (such as samples of yeasts or bacteria); or cell fractions, fragments or organelles (such as obtained by lysing cells and separating the components thereof by centrifugation or otherwise). Other examples of biological samples include blood, serum, urine, semen, fecal matter, cerebrospinal fluid, interstitial fluid, mucous, tears, sweat, pus, biopsied tissue (*e.g.*, obtained by a surgical biopsy or needle biopsy), nipple aspirates, milk, vaginal fluid, saliva, swabs (such as buccal swabs), or any material containing biomolecules that is derived from a first biological sample.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

[0041] Provided herein are compounds, compositions, kits, uses, and methods for treating neuropathies, including peripheral demyelinating neuropathies and hereditary peripheral demyelinating neuropathies (*e.g.*, Charcot-Marie-Tooth disease), by administering a compound that interacts with heat shock protein 90 (HSP90). Compounds that interact with HSP90 include inhibitors of HSP90 (*e.g.*, BIIB021, AUY922), modulators of HSP90, binders of HSP90, and compounds that modify HSP90. Compounds that interact with HSP90 include compounds that interact via non-covalent interactions or form covalent attachments to HSP90, or both. A compound that modifies HSP90 may change the structure or composition of HSP90, or both.

[0042] Hereditary neuropathies may be caused by defective myelin in the myelin sheath of nerve cells. Defects may be a result of demyelination, that is, loss of or damage to

myelin in the myelin sheath of nerve cells. Demyelination in certain disorders is associated with the misexpression (*e.g.*, overexpression) of peripheral myelin protein 22 (PMP22), which may result in protein misfolding and aggregation. Some demyelinating neuropathies are associated with an extra copy of the *PMP22* gene, which may lead to overexpression of the PMP22 protein. As a result, a high proportion of synthesized PMP22 may form cytosolic aggregates or be degraded by the proteasome, leaving insufficient properly folded PMP22 to be transported to the plasma membrane. Activation of the heat shock (HS) response, or chaperone response, may alleviate this insufficiency of PMP22, by aiding protein folding, preventing the formation of protein aggregates, enhancing the degradation of misfolded proteins or aggregates, and/or promoting transport of properly folded protein to the plasma membrane.

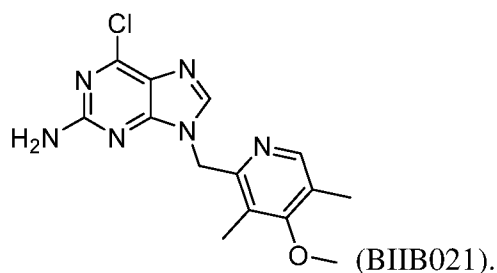
[0043] Inhibition of heat shock protein 90 (HSP90) may lead to upregulation of HSPs, by a pathway referred to as the heat shock (HS) response. Initiation of the HS response involves heat shock factor 1 (HSF1), which forms a complex with chaperones HSP40, HSP70, and HSP90. The complex inactivates HSF1 by preventing the monomeric form from trimerizing and translocating to the nucleus. HSF1 may disassociate from the complex as a result of cellular stress (*e.g.*, heat shock). Once in the nucleus HSF1 trimers may bind to heat shock elements (HSEs) of DNA, which initiates transcription of various heat shock genes, resulting in the upregulation of heat shock proteins in the cell. Without wishing to be bound by any particular theory, the interaction of an inhibitor, modulator, binder, or modifier with HSP90 may induce the HS response by disrupting the ability of HSP90 to complex with HSF1. Thus an HSP90 inhibitor, modulator, binder, or modifier can increase the availability of HSF1 trimers in the nucleus, wherein those trimers bind to HSEs, and lead to increased translation of HS genes and upregulation of HSPs.

[0044] Certain chaperone proteins formed as a result of the heat shock response may increase the flux of properly folded PMP22 to the plasma membrane. For example, chaperones may assist the folding of PMP22 leading to fewer misfolded or aggregated proteins. Chaperones may also assist in the degradation of PMP22 aggregates (*e.g.*, by the proteasome). The decrease in PMP22 aggregation and/or promotion of correct PMP22 folding may improve the flux of properly folded PMP22 transported to the plasma membrane. Without sufficient PMP22 the myelin formed at the plasma membrane will be defective. Administration of an HSP90 inhibitor, therefore, can help repair or improve the formation of myelin in the myelin sheath of nerve cells in a subject or biological sample, through the action of the heat shock protein pathway.

Useful Compounds

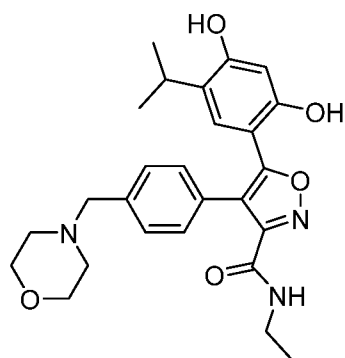
[0045] The methods and compositions described herein utilize a compound that interacts with HSP90 in the treatment of neuropathies. As described herein, the therapeutic effect may be a result of inhibition, modulation, binding, or modification of HSP90 by the compound. The compound may be provided for any composition, kit, or method described herein as a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. Any compound known in the art to inhibit, modulate, bind to, or modify HSP90 is contemplated in the invention described herein. In certain embodiments the compound is an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments the compound is an HSP90 modulator, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments the compound is an HSP90 binder, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments the compound is an HSP90 modifier, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof.

[0046] In certain embodiments, the HSP90 inhibitor is BIIB021. The name BIIB021 (Chemical Abstracts Service No. 848695-25-0), refers to a compound represented by the formula:



[0047] Methods of synthesis and characterization data for BIIB021, and related analogs, are provided in U.S. Patent No. 7,138,401, which is incorporated herein by reference in its entirety. BIIB021 may be provided for any composition, kit, or method described herein as a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof.

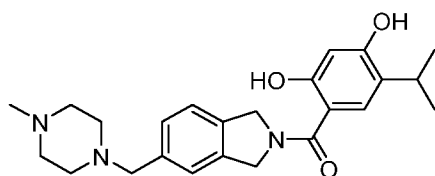
[0048] In certain embodiments, the HSP90 inhibitor is AUY922. The name AUY922 (Chemical Abstracts Service No. 747412-49-3), sometimes referred to as AUY-922, NVP-AUY922 or luminespib, refers to a compound represented by the formula:



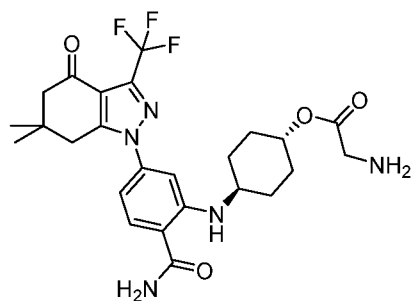
(AUY922).

[0049] Methods of synthesis and characterization data for AUY922, and related analogs, are provided in U.S. Patent No. 7,705,027, which is incorporated herein by reference in its entirety. AUY922 may be provided for any composition, kit, or method described herein as a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof.

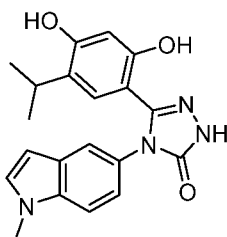
[0050] In certain embodiments, the HSP90 inhibitor is AT-13387, SNX-5422 (PF-04929113), or STA-9090 (ganetespib), which are represented by the following structures, as indicated:



(AT-13387),



(SNX-5422),



(STA-9090),

or pharmaceutically acceptable salts, tautomers, stereoisomers, solvates, hydrates, polymorphs, prodrugs, or compositions thereof.

[0051] In certain embodiments, the HSP90 inhibitor is alvespimycin hydrochloride (17-DMAG), ansamycin, elesclomol (STA-4783), gamitrinib, gedunin, geldanamycin, herbimycin A, macbecin I, novobiocin, radicicol, retaspimycin hydrochloride, tanespimycin

hydrochloride (17-AAG), 17-GMB-APA-GA, CH518303, CCT018159, CUDC-305, EC144, HSP990, KW-2478, MKT-077, MPC-3100, NMS-E973, NVP-BEP800, PU-29F, PU-H71, TAS-116, TRC-051384, SNX-2112, VER-50589, VER-155008, or XL-888, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. Structural formulae for these inhibitors are provided in *Table 1*.

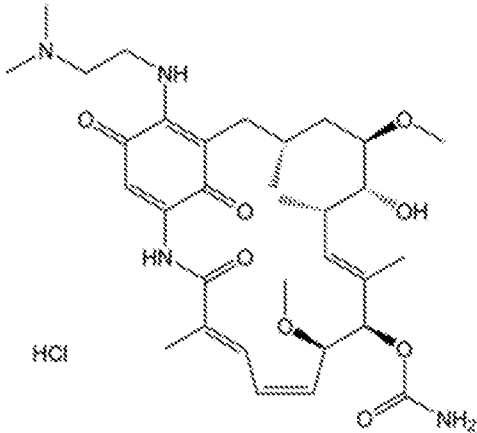
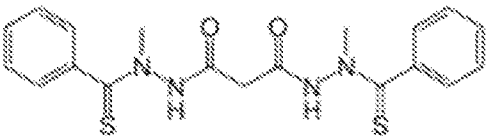
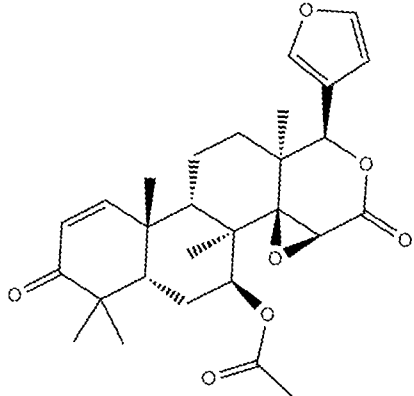
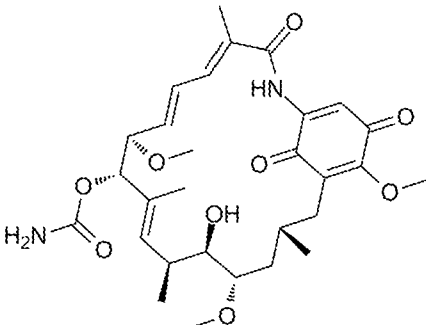
Table 1. Structures of HSP90 inhibitors	
Name	Structure
alvespimicyin hydrochloride (17-DMAG)	
elesclomol (STA-4783)	
gedunin	
geldanamycin	

Table 1. Structures of HSP90 inhibitors

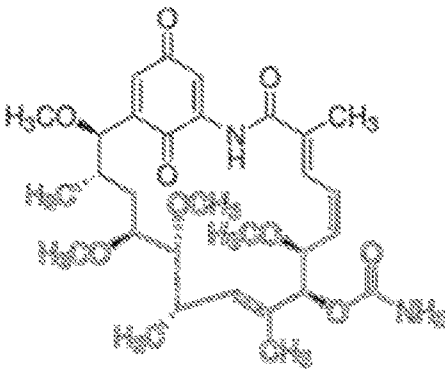
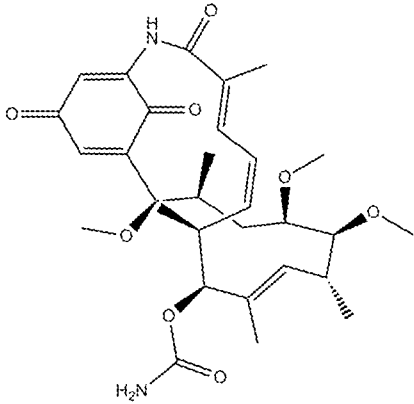
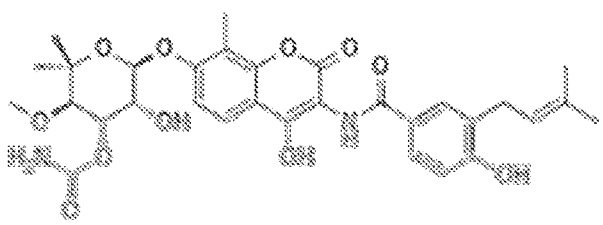
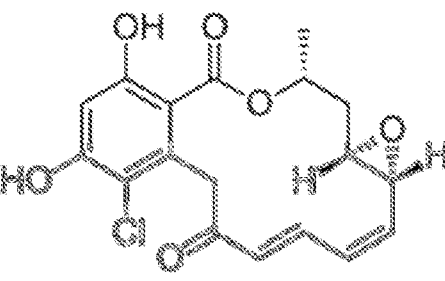
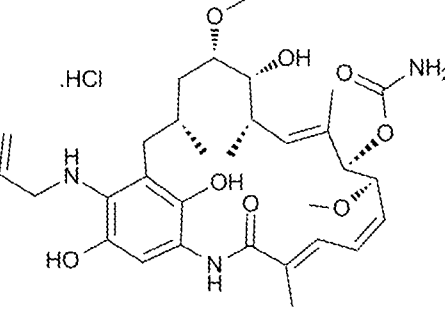
Name	Structure
herbimycin A	
macbecin I	
novobiocin	
radicicol	
retaspimycin hydrochloride (IPI-504)	

Table 1. Structures of HSP90 inhibitors

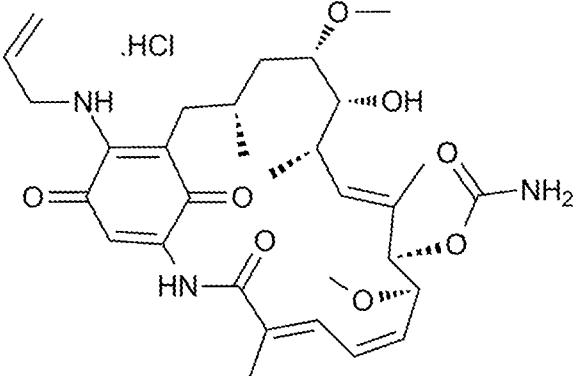
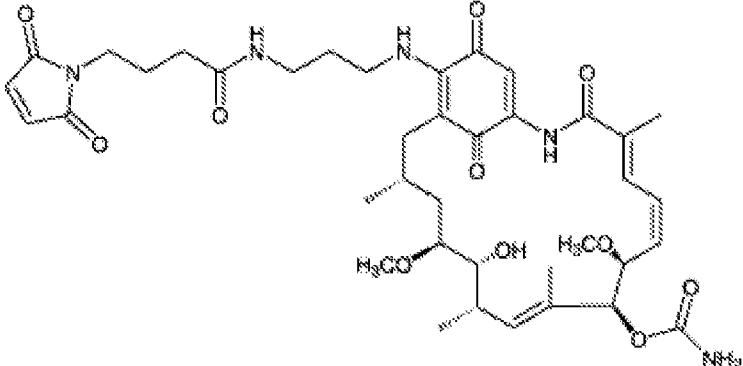
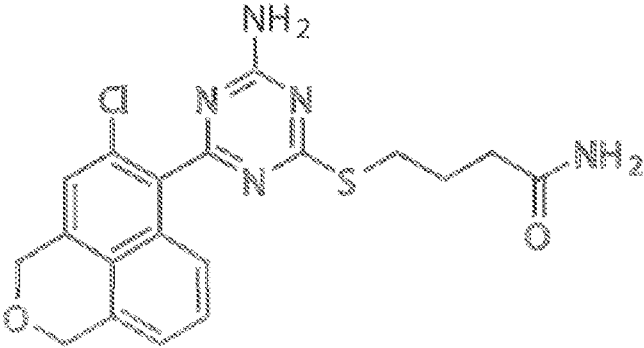
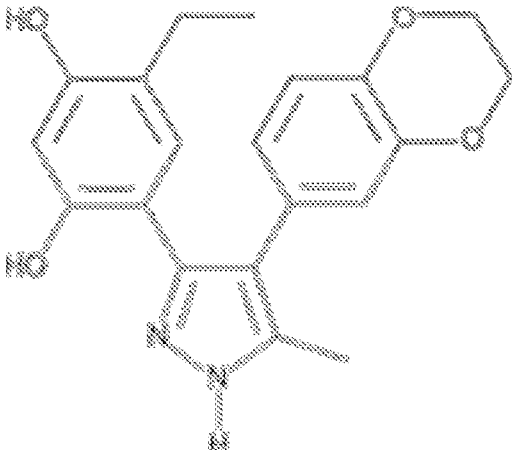
Name	Structure
tanespimycin hydrochloride (17-AAG)	
17-GMB-APA-GA	
CH518303	
CCT018159	

Table 1. Structures of HSP90 inhibitors

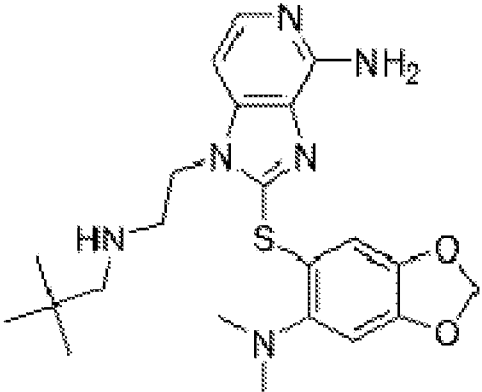
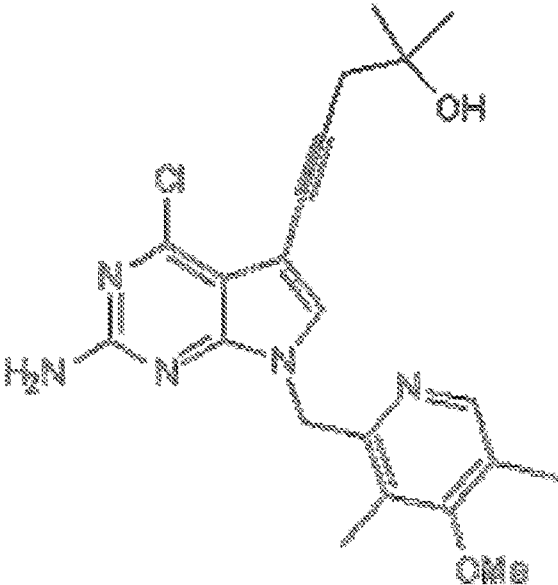
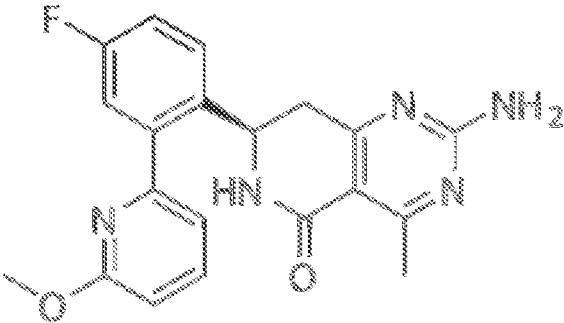
Name	Structure
CUDC-305	 The chemical structure of CUDC-305 features a central 1,2,4-triazole ring. At position 4, it is substituted with a 2-amino-3-pyridyl group. At position 5, it is substituted with a (tert-butylamino)methyl group. At position 3, it is substituted with a 2-methyl-6-methoxy-3,4-dihydro-2H-benzofuran-5-ylthio group.
EC144	 The chemical structure of EC144 consists of a 2-amino-4-chloro-1H-imidazo[5,1-b]pyridine core. At position 3 of the imidazole ring, there is a (2-methoxy-6-methylpyrimidin-4-yl)methyl substituent. At position 7 of the pyridine ring, there is a (2-hydroxy-2-methylpropyl)ethynyl substituent.
HSP990	 The chemical structure of HSP990 is a complex molecule featuring a 2-amino-4-methyl-6-oxo-1,2,3,4-tetrahydropyrimidin-5-yl group. This is linked via a methylene bridge to a 2-((4-fluorophenyl)(4-methoxypyridin-2-yl)methyl)amino group. The amide nitrogen is also linked to a methylene bridge that connects to the 4-methyl-6-oxo-1,2,3,4-tetrahydropyrimidin-5-yl group.

Table 1. Structures of HSP90 inhibitors

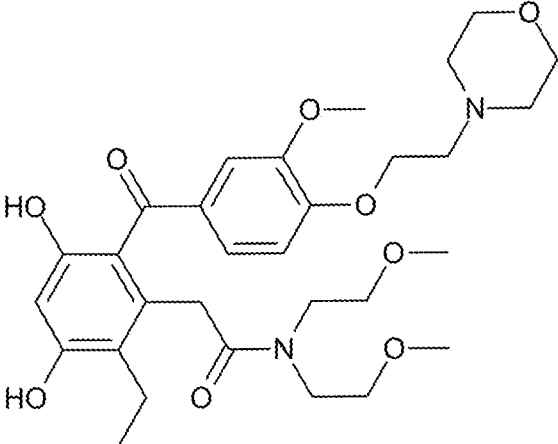
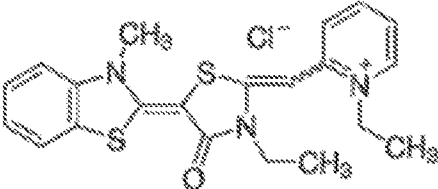
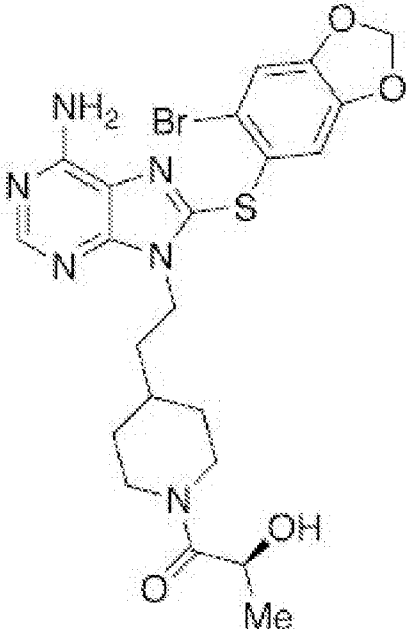
Name	Structure
KW-2478	 Chemical structure of KW-2478, an HSP90 inhibitor. It features a central benzene ring substituted with a hydroxyl group, a ketone, and two ether-linked side chains. One side chain is a 2-(2-methoxyethoxy)ethyl group, and the other is a 2-(2-methoxyethoxy)ethyl group attached to a morpholine ring.
MKT-077	 Chemical structure of MKT-077, an HSP90 inhibitor. It is a thiazolidine derivative with a benzothiazole moiety, a methyl group, and a side chain containing a pyridinium ring and a chloride counterion.
MPC-3100	 Chemical structure of MPC-3100, an HSP90 inhibitor. It is a complex molecule featuring a pyrimidine ring, a bromine atom, a furan ring, and a side chain containing a piperidine ring and a chiral center with a hydroxyl group and a methyl group.

Table 1. Structures of HSP90 inhibitors

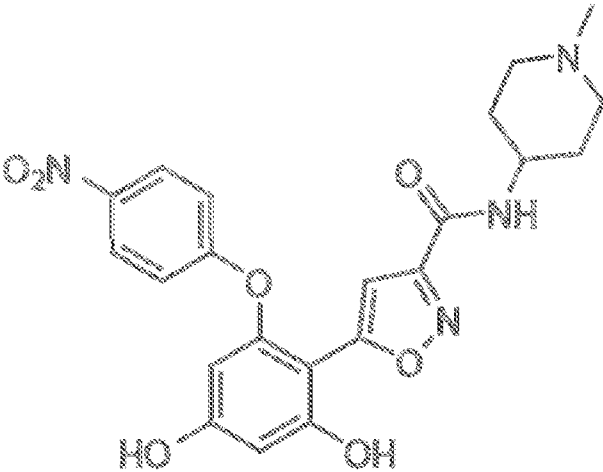
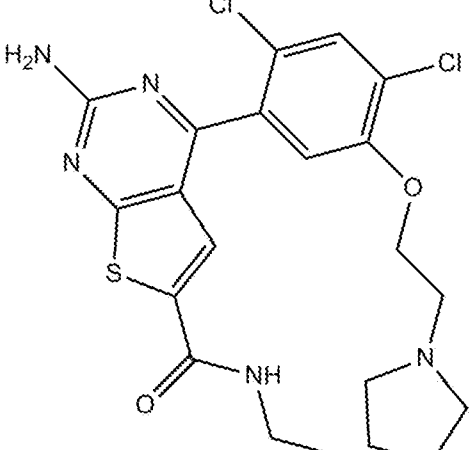
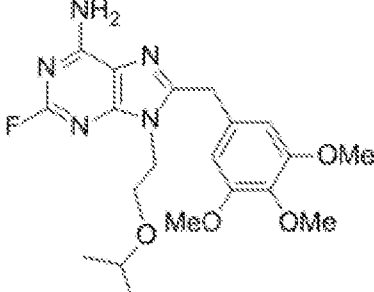
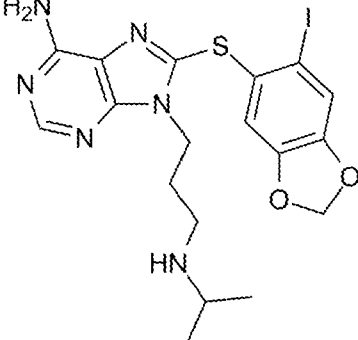
Name	Structure
NMS-E973	
NVP-BEP800	
PU-29F	
PU-H71	

Table 1. Structures of HSP90 inhibitors

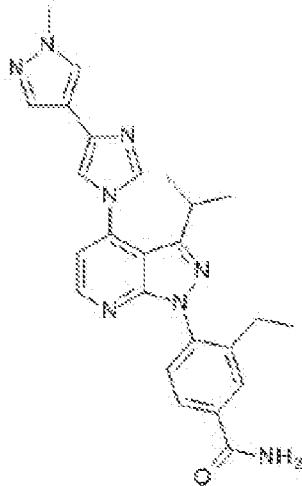
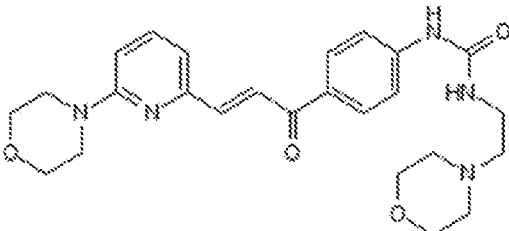
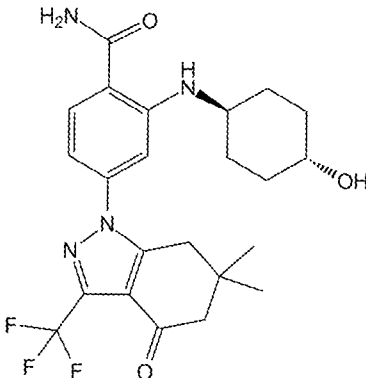
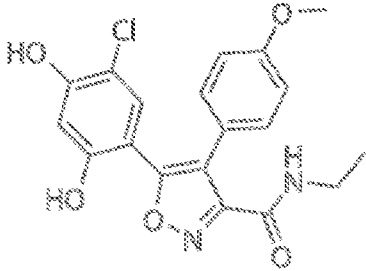
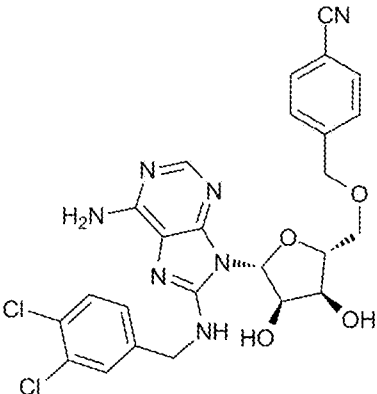
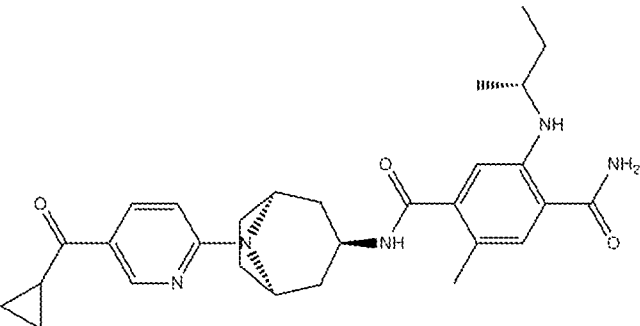
Name	Structure
TAS-116	
TRC-051384	
SNX-2112	
VER-50589	

Table 1. Structures of HSP90 inhibitors	
Name	Structure
VER-155008	 The chemical structure of VER-155008 is a complex molecule. It features a central 1,2,4-triazole ring. Attached to the triazole are a 4-amino-2-chlorophenyl group, a 4-chlorophenyl group, and a 4-cyano-2-methoxyphenyl group. The triazole ring is also connected to a 1,3-dihydroxy-2-methyl-5-oxo-1,3-dihydro-2H-pyrazole-4-carboxamide moiety.
XL-888	 The chemical structure of XL-888 is a complex molecule. It features a central 1,2,4-triazole ring. Attached to the triazole are a 4-amino-2-chlorophenyl group, a 4-chlorophenyl group, and a 4-cyano-2-methoxyphenyl group. The triazole ring is also connected to a 1,3-dihydroxy-2-methyl-5-oxo-1,3-dihydro-2H-pyrazole-4-carboxamide moiety.

[0052] In certain embodiments, the HSP90 inhibitor is EC1, EC42, EC74, EC75, EC82, EC102, EC114, EC115, EC116, EC119, EC127, EC137, EC139, EC141, EC142, or EC144, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. See, *e.g.*, Yun *et al.*, *J Immunol.* (2011) 186:563-575; Rangaraju *et al.*, *Neurobiology of Disease* (2008) 32:105-115; Dickey *et al.* *J. Clin. Invest.* (2007) 117:648-658; Dickey *et al.*, *The FASEB Journal* (2006) 20:753-755; Dickey *et al.*, *Curr. Alzh. Res.* (2005) 2:231-238. In certain embodiments, the compound is not EC116 or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, the HSP90 inhibitor is EC137 or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof.

Pharmaceutical Compositions, Kits, and Administration

[0053] The present invention provides pharmaceutical compositions comprising a compound that interacts with HSP90. The compound may be an inhibitor of HSP90 (*e.g.*, BIIB021, AUY922), modulator of HSP90, binder of HSP90, or a compound that modifies HSP90. In certain embodiments, the compound is an HSP90 inhibitor, or pharmaceutically

acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, or prodrug thereof, and optionally a pharmaceutically acceptable excipient. In some embodiments, the composition comprises an HSP90 inhibitor or a pharmaceutically acceptable salt thereof. In certain embodiments, the pharmaceutical composition comprises BIIB021, or pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, the pharmaceutical compositions comprises AUY922, or pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, the pharmaceutical composition comprises AT-13387, SNX-5422 (PF-04929113), or STA-9090 (ganetespib). In certain embodiments, the pharmaceutical composition comprises alvespimycin hydrochloride (17-DMAG), ansamycin, elesclomol (STA-4783), gamitrinib, gedunin, geldanamycin, herbimycin A, macbecin I, novobiocin, radicicol, retaspimycin hydrochloride, tanespimycin hydrochloride (17-AAG), 17-GMB-APA-GA, CH518303, CCT018159, CUDC-305, EC144, HSP990, KW-2478, MKT-077, MPC-3100, NMS-E973, NVP-BEP800, PU-29F, PU-H71, TAS-116, TRC-051384, SNX-2112, VER-50589, VER-155008, or XL-888. In certain embodiments, the HSP90 inhibitor is provided in an effective amount in the pharmaceutical composition. In some embodiments, the effective amount is a therapeutically effective amount. In some embodiments, the effective amount is a prophylactically effective amount.

[0054] The pharmaceutical compositions described herein are for use in treating a subject with a neuropathy. In certain embodiments, the neuropathy is a peripheral demyelinating neuropathy. In certain embodiments, the neuropathy is a hereditary neuropathy. In certain embodiments, the neuropathy is Charcot-Marie-Tooth disease. In certain embodiments, the neuropathy is caused by diabetes, impaired glucose tolerance, or Lyme disease. In certain embodiments, the neuropathy is associated with defective myelin in the myelin sheath of nerve cells. In certain embodiments, the neuropathy is associated with axonal shrinkage and atrophy. In certain embodiments, the neuropathy is associated with overexpression of PMP22. In certain embodiments, the neuropathy is associated with misfolding or aggregation of peripheral myelin protein 22 (PMP22). In some embodiments, the neuropathy is associated with an accumulation of cytosolic aggregates of peripheral myelin protein 22 (PMP22). In some embodiments, the neuropathy is ameliorated by activation of the heat shock protein response. In certain embodiments, the neuropathy is anti-MAG peripheral neuropathy, Charcot-Marie-Tooth disease (CMT) (*e.g.*, CMT1A, Dejerine-Sottas disease, congenital hypomyelinating neuropathy (CHN), Russe-type hereditary motor and sensor neuropathy (HMSNR), CMT with pyramidal features, CMT with optic atrophy,

Cowchock syndrome, Rosenberg-Chutorian syndrome, Roussy-Lévy syndrome), chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain-Barré syndrome, hereditary neuropathy with liability to pressure palsy (HNPP), or progressive inflammatory neuropathy. In certain embodiments, the neuropathy is a chemotherapy-induced peripheral neuropathy (CIPN).

[0055] In certain embodiments, the compound or pharmaceutical composition is a solid. In certain embodiments, the compound or pharmaceutical composition is a powder. In certain embodiments, the compound or pharmaceutical composition can be dissolved in a liquid to make a solution. In certain embodiments, the compound or pharmaceutical composition is dissolved in water to make an aqueous solution. In certain embodiments, the pharmaceutical composition is a liquid for parental injection. In certain embodiments, the pharmaceutical composition is a liquid for oral administration (*e.g.*, ingestion). In certain embodiments, the pharmaceutical composition is a liquid (*e.g.*, aqueous solution) for intravenous injection. In certain embodiments, the pharmaceutical composition is a liquid (*e.g.*, aqueous solution) for subcutaneous injection.

[0056] After formulation with an appropriate pharmaceutically acceptable excipient in a desired dosage, the pharmaceutical compositions of this invention can be administered to humans and other animals orally, parenterally, intracisternally, intraperitoneally, topically, buccally, or the like, depending on the disease or condition being treated. In certain embodiments, a pharmaceutical composition comprising an HSP90 inhibitor is administered, orally or parenterally, at dosage levels of each pharmaceutical composition sufficient to deliver from about 0.001 mg/kg to about 200 mg/kg in one or more dose administrations for one or several days (depending on the mode of administration). In certain embodiments, the effective amount per dose varies from about 0.001 mg/kg to about 200 mg/kg, about 0.001 mg/kg to about 100 mg/kg, about 0.01 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, preferably from about 0.1 mg/kg to about 40 mg/kg, preferably from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic and/or prophylactic effect. In certain embodiments, the compounds described herein may be at dosage levels sufficient to deliver from about 0.001 mg/kg to about 200 mg/kg, about 0.001 mg/kg to about 100 mg/kg, about 0.01 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, preferably from about 0.1 mg/kg to about 40 mg/kg, preferably from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about 0.1 mg/kg to about 10 mg/kg, and more preferably

from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic and/or prophylactic effect. The desired dosage may be delivered three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks. In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations). In certain embodiments, each composition described herein is administered at a dose that is below the dose at which the agent causes non-specific effects.

[0057] In certain embodiments, the pharmaceutical composition is administered at a dose of about 0.001 mg to about 200 mg a day. In certain embodiments, the pharmaceutical composition is administered at a dose of about 0.01 mg to about 100 mg a day. In certain embodiments, pharmaceutical composition is administered at a dose of about 0.01 mg to about 50 mg a day. In certain embodiments, the pharmaceutical composition is administered at a dose of about 0.01 mg to about 10 mg a day. In certain embodiments, the pharmaceutical composition is administered at a dose of about 0.1 mg to about 10 mg a day.

[0058] Pharmaceutical compositions described herein can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the steps of bringing the composition comprising an HSP90 inhibitor, into association with a carrier and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0059] Pharmaceutical compositions can be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” is a discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[0060] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition of the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100% (w/w) active ingredient.

[0061] Pharmaceutically acceptable excipients used in the manufacture of provided pharmaceutical compositions include inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives,

buffering agents, lubricating agents, and/or oils. Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and perfuming agents may also be present in the composition.

[0062] Exemplary diluents include calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, and mixtures thereof.

[0063] Exemplary granulating and/or dispersing agents include potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose, and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, and mixtures thereof.

[0064] Exemplary surface active agents and/or emulsifiers include natural emulsifiers (*e.g.* acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (*e.g.* bentonite (aluminum silicate) and Veegum (magnesium aluminum silicate)), long chain amino acid derivatives, high molecular weight alcohols (*e.g.* stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (*e.g.* carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (*e.g.* carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (*e.g.* polyoxyethylene sorbitan monolaurate (Tween 20), polyoxyethylene sorbitan (Tween 60), polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monopalmitate (Span 40), sorbitan monostearate (Span 60), sorbitan tristearate (Span 65), glyceryl monooleate, sorbitan monooleate (Span 80)), polyoxyethylene esters (*e.g.* polyoxyethylene monostearate (Myrj 45), polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (*e.g.* Cremophor™), polyoxyethylene ethers, (*e.g.* polyoxyethylene lauryl ether (Brij 30)), poly(vinyl-pyrrolidone), diethylene glycol

monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, Pluronic F-68, Poloxamer-188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, and/or mixtures thereof.

[0065] Exemplary binding agents include starch (*e.g.* cornstarch and starch paste), gelatin, sugars (*e.g.* sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol, *etc.*), natural and synthetic gums (*e.g.* acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (Veegum), and larch arabogalactan), alginates, polyethylene oxide, polyethylene glycol, inorganic calcium salts, silicic acid, polymethacrylates, waxes, water, alcohol, and/or mixtures thereof.

[0066] Exemplary preservatives include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives. In certain embodiments, the preservative is an antioxidant. In other embodiments, the preservative is a chelating agent.

[0067] Exemplary antioxidants include alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite.

[0068] Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA) and salts and hydrates thereof (*e.g.*, sodium edetate, disodium edetate, trisodium edetate, calcium disodium edetate, dipotassium edetate, and the like), citric acid and salts and hydrates thereof (*e.g.*, citric acid monohydrate), fumaric acid and salts and hydrates thereof, malic acid and salts and hydrates thereof, phosphoric acid and salts and hydrates thereof, and tartaric acid and salts and hydrates thereof. Exemplary antimicrobial preservatives include benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and thimerosal.

[0069] Exemplary antifungal preservatives include butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid.

[0070] Exemplary alcohol preservatives include ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and phenylethyl alcohol.

[0071] Exemplary acidic preservatives include vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and phytic acid.

[0072] Other preservatives include tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, Glydant Plus, Phenonip, methylparaben, Germall 115, Germaben II, Neolone, Kathon, and Euxyl.

[0073] Exemplary buffering agents include citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, and mixtures thereof.

[0074] Exemplary lubricating agents include magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, and mixtures thereof.

[0075] Exemplary natural oils include almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mint, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary

synthetic oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and mixtures thereof.

[0076] Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active agents, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents. In certain embodiments for parenteral administration, agents of the invention are mixed with solubilizing agents such as CREMOPHOR EL[®] (polyethoxylated castor oil), alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and combinations thereof.

[0077] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions, may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. Sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

[0078] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[0079] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active agent is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium

phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

[0080] Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

[0081] The active agents can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active agent may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, *e.g.*, tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

[0082] Formulations suitable for topical administration include liquid or semi-liquid preparations such as liniments, lotions, gels, applicants, oil-in-water or water-in-oil emulsions such as creams, ointments, or pastes; or solutions or suspensions such as drops. Formulations for topical administration to the skin surface can be prepared by dispersing the drug with a dermatologically acceptable carrier such as a lotion, cream, ointment, or soap. Useful carriers are capable of forming a film or layer over the skin to localize application and inhibit removal. For topical administration to internal tissue surfaces, the agent can be dispersed in a liquid tissue adhesive or other substance known to enhance adsorption to a tissue surface. For example, hydroxypropylcellulose or fibrinogen/thrombin solutions can be used to advantage. Alternatively, tissue-coating solutions, such as pectin-containing formulations can be used. Ophthalmic formulation, ear drops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of an agent to the body. Such dosage forms can be made by dissolving or dispensing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the agent across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the agent in a polymer matrix or gel.

[0083] Additionally, the carrier for a topical formulation can be in the form of a hydroalcoholic system (*e.g.*, quids and gels), an anhydrous oil or silicone based system, or an emulsion system, including, but not limited to, oil-in-water, water-in-oil, water-in-oil-in-water, and oil-in-water-in-silicone emulsions. The emulsions can cover a broad range of consistencies including thin lotions (which can also be suitable for spray or aerosol delivery), creamy lotions, light creams, heavy creams, and the like. The emulsions can also include microemulsion systems. Other suitable topical carriers include anhydrous solids and semisolids (such as gels and sticks); and aqueous based mousse systems.

[0084] It will also be appreciated that the compositions described herein can be employed in combination therapies, that is, the compounds and pharmaceutical compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder, or they may achieve different effects (*e.g.*, control of any adverse effects).

[0085] In addition to the HSP90 inhibitor, the pharmaceutical composition described herein may comprise one or more additional pharmaceutical agents. In certain embodiments, the additional pharmaceutical agent is an agent useful in the treatment of a neuropathy. In certain embodiments, the additional pharmaceutical agent is an agent that repairs or improves the formation of myelin in the myelin sheath of nerve cells. In certain embodiments, the additional pharmaceutical agent is an agent that activates the heat shock protein response. In certain embodiments, the additional pharmaceutical agent is ascorbic acid, a progesterone antagonist, curcumin, an activator of autophagy (*e.g.*, rapamycin), baclofen, naltrexone, sorbitol, or a mixture (PXT3003) of baclofen, naltrexone, and sorbitol. In certain embodiments, the additional pharmaceutical agent is an HSP90 inhibitor. In some embodiments, the additional pharmaceutical agent is BIIB021 or AUY922, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, or prodrug thereof. In some embodiments, the additional pharmaceutical agent is AT-13387, SNX-5422 (PF-04929113), or STA-9090 (ganetespib), or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, or prodrug thereof. In certain embodiments, the additional pharmaceutical agent is alvespimycin hydrochloride (17-DMAG), ansamycin, elesclomol (STA-4783), gamitrinib, gedunin, geldanamycin, herbimycin A, macbecin I, novobiocin, radicicol, retaspimycin hydrochloride, tanespimycin hydrochloride (17-AAG), 17-GMB-APA-GA, CH518303, CCT018159, CUDC-305, EC144, HSP990, KW-2478, MKT-077, MPC-3100, NMS-E973, NVP-BEP800, PU-29F, PU-H71, TAS-116, TRC-051384, SNX-2112, VER-50589, VER-155008, or XL-888, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, or prodrug thereof.

[0086] In another aspect, provided are kits (*e.g.*, pharmaceutical packs) for treating and/or preventing a pathological condition of a subject. The pharmaceutical compositions comprise a compound that interacts with HSP90. The compound may be an inhibitor of HSP90 (*e.g.*, BIIB021, AUY922), modulator of HSP90, binder of HSP90, or a compound that modifies HSP90. In certain embodiments, the kit may comprise an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof, and a container (*e.g.*, a vial, ampule, bottle, syringe, and/or dispenser package, or other suitable container). In certain embodiments, the kit includes a first container comprising an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof; and instructions for administering the HSP90 inhibitor, or a pharmaceutically acceptable salt,

tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof, to the subject to treat and/or prevent the pathological condition. In certain embodiments, the kit comprises a pharmaceutical composition comprising an HSP90 inhibitor, and instructions for the administration of the pharmaceutical compositions to a subject. In some embodiments, the HSP90 inhibitor is BIIB021, or pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the HSP90 inhibitor is AUY922, or pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the HSP90 inhibitor is AT-13387, SNX-5422 (PF-04929113), or STA-9090 (ganetespib), or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, the HSP90 inhibitor is alvespimycin hydrochloride (17-DMAG), ansamycin, elesclomol (STA-4783), gamitrinib, gedunin, geldanamycin, herbimycin A, macbecin I, novobiocin, radicicol, retaspimycin hydrochloride, tanespimycin hydrochloride (17-AAG), 17-GMB-APA-GA, CH518303, CCT018159, CUDC-305, EC144, HSP990, KW-2478, MKT-077, MPC-3100, NMS-E973, NVP-BEP800, PU-29F, PU-H71, TAS-116, TRC-051384, SNX-2112, VER-50589, VER-155008, or XL-888, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, the kits of the present invention include one or more additional approved pharmaceutical agent(s). In certain embodiments, the instruction includes a notice in the form prescribed by a governmental agency such as the U.S. Food and Drug Administration (FDA) regulating the manufacture, use, or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use, or sale for human administration.

Methods of Treatment

[0087] Provided herein are methods for treating neuropathies. In certain embodiments, the neuropathy is a peripheral demyelinating neuropathy. In certain embodiments, the neuropathy is a hereditary neuropathy. In certain embodiments, the neuropathy is caused by diabetes, impaired glucose tolerance, or Lyme disease. In certain embodiments, the neuropathy is Charcot-Marie-Tooth disease. In certain embodiments, the neuropathy is associated with defective myelin in the myelin sheath of nerve cells. In certain embodiments, the neuropathy is associated with axonal shrinkage and atrophy. In certain embodiments, the neuropathy is associated with overexpression of peripheral myelin protein 22 (PMP22). In certain embodiments, the neuropathy is associated with misfolding or

aggregation of peripheral myelin protein 22 (PMP22). In some embodiments, the neuropathy is associated with an accumulation of cytosolic aggregates of peripheral myelin protein 22 (PMP22). In some embodiments, the neuropathy is ameliorated by activation of the heat shock protein response.

[0088] In certain embodiments, the method comprises administering a compound that interacts with HSP90. The compound may be an inhibitor of HSP90, a modulator of HSP90, a binder of HSP90, or a compound that modifies HSP90. In certain embodiments, the method comprises administering an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof, to a subject in need thereof. In some embodiments, the method comprises administering an HSP90 inhibitor or a pharmaceutically acceptable salt thereof. In some embodiments, the method comprises administering a pharmaceutical composition comprising an HSP90 inhibitor. In certain embodiments, the method comprises administering BIIB021, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the method comprises administering BIIB021, or a pharmaceutically acceptable salt thereof. In some embodiments, the method comprises administering a pharmaceutical composition comprising BIIB021. In certain embodiments, the method comprises administering the HSP90 inhibitor AUY922, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the method comprises administering AUY922 or a pharmaceutically acceptable salt thereof. In some embodiments, the method comprises administering a pharmaceutical composition comprising AUY922. In certain embodiments, the HSP90 inhibitor is AT-13387, SNX-5422 (PF-04929113), or STA-9090 (ganetespib), or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, the HSP90 inhibitor is alvespimycin hydrochloride (17-DMAG), ansamycin, elesclomol (STA-4783), gamitrinib, gedunin, geldanamycin, herbimycin A, macbecin I, novobiocin, radicicol, retaspimycin hydrochloride, tanespimycin hydrochloride (17-AAG), 17-GMB-APA-GA, CH518303, CCT018159, CUDC-305, EC144, HSP990, KW-2478, MKT-077, MPC-3100, NMS-E973, NVP-BEP800, PU-29F, PU-H71, TAS-116, TRC-051384, SNX-2112, VER-50589, VER-155008, or XL-888, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof.

[0089] The present invention also provides uses of an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph,

prodrug, or composition thereof, in the manufacture of a medicament for the treatment and/or prevention of a neuropathy. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922. In certain embodiments, the neuropathy is a peripheral demyelinating neuropathy. In certain embodiments, the neuropathy is a hereditary neuropathy. In certain embodiments, the neuropathy is Charcot-Marie-Tooth disease. In certain embodiments, the neuropathy is associated with defective myelin in the myelin sheath of nerve cells. In certain embodiments, the neuropathy is associated with axonal shrinkage and atrophy. In certain embodiments, the neuropathy is associated with overexpression of peripheral myelin protein 22 (PMP22). In certain embodiments, the neuropathy is associated with misfolding or aggregation of peripheral myelin protein 22 (PMP22). In some embodiments, the neuropathy is associated with an accumulation of cytosolic aggregates of PMP22 in Schwann cells. In certain embodiments, the neuropathy is ameliorated by activation of the heat shock protein response.

[0090] In certain embodiments, the methods of the invention comprise administering to the subject an effective amount of an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922. In some embodiments, the effective amount is a therapeutically effective amount.

[0091] In another aspect, the present invention provides methods for treatment of a neuropathy by administering a therapeutically effective amount of an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922. In certain embodiments, the neuropathy is a peripheral demyelinating neuropathy. In certain embodiments, the neuropathy is a hereditary neuropathy. In certain embodiments, the neuropathy is Charcot-Marie-Tooth disease. In certain embodiments, the neuropathy is associated with defective myelin in the myelin sheath of nerve cells. In certain embodiments, the neuropathy is associated with axonal shrinkage and atrophy. In certain embodiments, the neuropathy is associated with overexpression of peripheral myelin protein 22 (PMP22). In certain embodiments, the neuropathy is associated with misfolding or aggregation of peripheral myelin protein 22 (PMP22). In some embodiments, the neuropathy is associated with an accumulation of cytosolic aggregates of PMP22 in Schwann cells. In some embodiments, the neuropathy is ameliorated by activation of the heat shock protein response.

[0092] In any of the methods or uses described herein, the neuropathy may be a peripheral neuropathy or a neuropathy of the central nervous system, and may be hereditary or non-hereditary. In certain embodiments, the neuropathy is a peripheral demyelinating neuropathy. In certain embodiments, the neuropathy is anti-MAG peripheral neuropathy, Charcot-Marie-Tooth disease (CMT) (*e.g.*, CMT1A, Dejerine-Sottas disease, congenital hypomyelinating neuropathy (CHN), Russe-type hereditary motor and sensor neuropathy (HMSNR), CMT with pyramidal features, CMT with optic atrophy, Cowchock syndrome, Rosenberg-Chutorian syndrome, Roussy-Lévy syndrome), chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain-Barré syndrome, hereditary neuropathy with liability to pressure palsy (HNPP), or progressive inflammatory neuropathy. In certain embodiments, the neuropathy is a chemotherapy-induced peripheral neuropathy (CIPN).

[0093] In another aspect, the present invention provides methods of repairing or improving the formation of myelin in the myelin sheath of nerve cells in a subject by administering an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922.

[0094] In an additional aspect, the present invention provides methods of reducing misfolding or aggregation of peripheral myelin protein 22 (PMP22) in a subject by administering to a subject in need thereof an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922.

[0095] In an additional aspect, the present invention provides methods of reducing cytosolic aggregates of peripheral myelin protein 22 (PMP22) in a subject by administering to a subject in need thereof an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922.

[0096] In an additional aspect, the present invention provides methods of activating the heat shock protein response in a subject by administering to a subject in need thereof an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922.

[0097] In yet another aspect, the present invention provides methods of repairing or improving the formation of myelin in the myelin sheath in a biological sample by contacting a biological sample with an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922.

[0098] In an additional aspect, the present invention provides methods of reducing misfolding or aggregation of peripheral myelin protein 22 (PMP22) in a biological sample by contacting a biological sample with an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922.

[0099] In an additional aspect, the present invention provides methods of reducing cytosolic aggregates of peripheral myelin protein 22 (PMP22) in a biological sample by contacting a biological sample with an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922.

[00100] In an additional aspect, the present invention provides methods of activating the heat shock protein response in a biological sample by contacting a biological sample with an HSP90 inhibitor, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the inhibitor is BIIB021. In some embodiments, the inhibitor is AUY922.

[00101] In another aspect, the method of treating a neuropathy comprises the steps of determining if a subject exhibits overexpression of PMP22, and administering to the subject an HSP90 inhibitor (*e.g.*, BIIB021, AUY922), or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, method of treating a neuropathy comprises the steps of determining if a subject has more than one copy of the *PMP22* gene, and administering to the subject an HSP90 inhibitor (*e.g.*, BIIB021, AUY922), or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, the method of treating a neuropathy comprises the steps of performing a diagnostic test to determining if a subject exhibits misfolding or aggregation of peripheral myelin protein 22 (PMP22), and administering to the subject an HSP90 inhibitor (*e.g.*, BIIB021, AUY922), or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate,

hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the determination or diagnostic test comprises measuring PMP22 mRNA in a sample obtained from a Schwann cell. In some embodiments, the determination or diagnostic test comprises measuring PMP22 protein or aggregated PMP22 protein in a sample obtained from a Schwann cell. In some embodiments, the determination or diagnostic test comprises fluorescent *in situ* hybridization or genetic sequencing.

[00102] In certain embodiments, the subject described herein is a human. In certain embodiments, the subject is an animal. The animal may be of either sex and may be at any stage of development. In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a domesticated animal, such as a dog, cat, cow, pig, horse, sheep, or goat. In certain embodiments, the subject is a companion animal, such as a dog or cat. In certain embodiments, the subject is a livestock animal, such as a cow, pig, horse, sheep, or goat. In certain embodiments, the subject is a zoo animal. In another embodiment, the subject is a research animal such as a rodent (*e.g.*, mouse, rat), dog, pig, or non-human primate. In certain embodiments, the animal is a genetically engineered animal. In certain embodiments, the animal is a transgenic animal.

[00103] In certain embodiments, the biological sample described herein is one or more cells. In certain embodiments, the biological sample described herein is one or more cancer cells. In certain embodiments, a cell described herein is *in vitro*. In certain embodiments, a cell described herein is *ex vivo*. In certain embodiments, a cell described herein is *in vivo*. In certain embodiments, a cell described herein is a malignant cell. In certain embodiments, a cell In certain embodiments, the biological sample described herein is blood, bone, or tissue. In certain embodiments, the biological sample described herein is bone marrow or lymph node. In certain embodiments, the biological sample described herein is biopsied tissue. In certain embodiments, the biological sample described herein is a tumor.

[00104] Certain methods described herein may comprise administering one or more additional pharmaceutical agent(s) in combination with the compounds described herein. In certain embodiments, the additional pharmaceutical agent comprises an agent useful in the treatment of a neuropathy. In certain embodiments, the additional pharmaceutical agent comprises an agent that repairs or improves the formation of myelin in the myelin sheath of nerve cells. In certain embodiments, the additional pharmaceutical agent comprises an agent that activates the heat shock protein response. In certain embodiments, the additional pharmaceutical agent comprises an HSP90 inhibitor. In certain embodiments, the additional pharmaceutical agent comprises ascorbic acid, a progesterone antagonist, curcumin, an

activator of autophagy (*e.g.*, rapamycin), baclofen, naltrexone, sorbitol, or a mixture (PXT3003) of baclofen, naltrexone, and sorbitol. In some embodiments, the additional pharmaceutical agent is BIIB021 or AUY922, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In some embodiments, the additional pharmaceutical agent is AT-13387, SNX-5422 (PF-04929113), or STA-9090 (ganetespib), or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof. In certain embodiments, the additional pharmaceutical agent is alvespimycin hydrochloride (17-DMAG), ansamycin, elesclomol (STA-4783), gamitrinib, gedunin, geldanamycin, herbimycin A, macbecin I, novobiocin, radicicol, retaspimycin hydrochloride, tanespimycin hydrochloride (17-AAG), 17-GMB-APA-GA, CH518303, CCT018159, CUDC-305, EC144, HSP990, KW-2478, MKT-077, MPC-3100, NMS-E973, NVP-BEP800, PU-29F, PU-H71, TAS-116, TRC-051384, SNX-2112, VER-50589, VER-155008, or XL-888, or a pharmaceutically acceptable salt, tautomer, stereoisomer, solvate, hydrate, polymorph, prodrug, or composition thereof.

EXAMPLES

[00105] In order that the invention described herein may be more fully understood, the following examples are set forth. The synthetic and biological examples described in this application are offered to illustrate the compounds, pharmaceutical compositions, and methods provided herein and are not to be construed in any way as limiting their scope.

Introduction

[00106] The heat shock (HS) pathway represents a cellular stress response, which results in elevated expression of cytoprotective chaperones, or heat shock proteins (HSPs). Activation of chaperones has been shown to reduce the aggregation of misfolded proteins and alleviate disease phenotypes in various neurodegenerative models (Jiang *et al.*, 2013); (Lin *et al.*, 2013); (Kalmar *et al.*, 2014). It has been proposed that an increase in the availability of functional HSPs aids in the folding, disaggregation or enhanced degradation of misfolded proteins (Jinwal *et al.*, 2013); (Mattoo *et al.*, 2013); (Sancho *et al.*, 2014). Induction of the HS pathway can be achieved through inhibition of HSP90, which disrupts its interaction with Heat Shock Factor-1 (HSF-1) leading to transcriptional activation of the HS response (Ali *et al.*, 1998). Although HSP90 inhibitors have been investigated primarily for their anti-cancer properties, when used within a defined concentration window they can be beneficial in the treatment of protein misfolding disorders (Westerheide and Morimoto, 2005).

[00107] A number of CNS neurodegenerative disorders involve protein misfolding and are targets for chaperone therapy. Charcot-Marie-Tooth disease type 1A (CMT1A) is a hereditary peripheral demyelinating neuropathy which most often is associated with misexpression of peripheral myelin protein 22 (PMP22), an aggregation prone Schwann cell protein (Lupski and Garcia, 1992). Transgenic animal models, such as the C22 mouse, reproduce most of the CMT1A phenotypic traits, including demyelination of peripheral nerves, reduced nerve conduction velocity, impaired locomotor performance and age-associated disease progression (Huxley *et al.*, 1996); (Passage *et al.*, 2004); (Chittoor *et al.*, 2013). This mouse model carries 7 copies of the human *PMP22* gene and expresses about 1.7 times more human *PMP22* mRNA as compared to the endogenous mouse mRNA (Huxley *et al.*, 1996). A previous study showed that activation of the HS pathway using EC137, a synthetic HSP90 inhibitor, reduced the aggregation of PMP22 and improved myelination in neuron-glia explant cultures from C22 mice (Rangaraju *et al.*, 2008). In an *in vivo* study of neuropathic Trembler J mice it was found that an increase in chaperone expression through intermittent fasting supported maintenance of nerve myelin and locomotor performance (Madorsky *et al.*, 2009). In accordance, enhancement of the stress response by life-long calorie restriction was beneficial for peripheral nerve integrity in aged rats (Rangaraju *et al.*, 2008); (Opalach *et al.*, 2010). In addition, induction of chaperones in an induced-diabetic peripheral neuropathy model reversed the associated sensory deficits (Urban *et al.*, 2012). Therefore a number of experimental scenarios support the notion that chaperones are important in supporting myelin and peripheral nerve function.

[00108] In these examples, five commercially available HSP90 inhibitors were screened and NVP-AUY922 (referred to as AUY922 from here on) was identified as an effective compound in improving *in vitro* myelination in explant cultures from C22 mice. This positive response correlated with robust induction of chaperones in Schwann cells, in a dose- and time-dependent manner. *In vivo* administration of AUY922 to a cohort of neuropathic C22 mice attenuated the decline in their neuromuscular performance and preserved peripheral nerve morphology.

Materials and Methods

Mouse colonies and genotyping

[00109] A founder pair of C22 mice were bred on C57Bl/6 background for multiple generations (Huxley *et al.*, 1996). For effective breeding, affected females were bred to wild type C57Bl/6 mice obtained from Jackson laboratories. All animals were maintained under

SPF conditions within the University of Florida animal care facilities and strictly in compliance with procedures approved by the University of Florida Institutional Animal Care and Use Committee. For genotyping, DNA was obtained from tail biopsies of less than 8-day old pups and analyzed by PCR using the following primer sets: C22- 5'

TTCTGCTGCCTGTGAGGAC 3' (SEQ ID NO: 3) and 5'

GGGTGAAGAGTTGGCAGAAG 3' (SEQ ID NO: 4) which yield a 209 base pair product.

The endogenous mouse PMP22 was identified using the following primers: 5'

GGAGTGACACCCAGAGGGTA 3' (SEQ ID NO: 5) and 5'

CCTCACCACTCCCTGGTAAA 3' (SEQ ID NO: 6) yielding a 248 base pair product. At weaning age, littermates were segregated by genotype and gender, and randomly assigned to vehicle and AUY922 treatment groups. All efforts were made to reduce the number of animals used and to minimize their discomfort.

Cell culture models

[00110] Non-myelinating Schwann cells were established from the sciatic nerves of postnatal day 2 rats (Notterpek *et al.*, 1999). The cells were maintained in DMEM (Gibco) and supplemented with 10% FCS (HyClone), 100 µg/ml bovine pituitary extract (Biomedical Technologies Inc.) and 5 µM forskolin (Calbiochem). Dorsal-Root Ganglion (DRG) explants were established from embryonic day 12-13 Wt and C22 embryos (Rangaraju *et al.*, 2008). Briefly, DRGs were dissociated in 0.25% trypsin (Gibco) and plated onto collagen-coated cell culture wells. DNA isolated from each embryo was used for genotyping, as described above. All explants were maintained in MEM (Gibco), 10% FCS (Hyclone), 0.3% glucose (Sigma-Aldrich), 5 10 mM HEPES (Gibco) and 100 ng/ml nerve growth factor (Harlan Bioproducts for Science) for 7 days. The cultures were then supplemented with 50 µg/ml ascorbate for an additional 7 days to promote myelin formation. For Schwann cell-depleted neuronal cultures, explants were subjected to alternate-day treatment with 5-fluoro-2'-deoxyuridine (FUdR) for 10 days and then continued on the same paradigm described above (Rangaraju *et al.*, 2008).

Pharmacologic treatment paradigms

[00111] HSP90 inhibitor compounds, including AT13387 (S1163), AUY922 (S1069), BIIB021 (S1175), SNX5422 (S2656), STA9090 (S1159), were purchased from Selleckchem and stored at a stock concentration of 1 mM in DMSO.

[00112] Primary Schwann cells were treated with HSP90 inhibitors at the indicated concentrations in complete media, as described herein, 24 h after seeding. DMSO served as the vehicle control while geldanamycin (GA) was used as a positive control for heat shock pathway activation.

[00113] The DRG explant cultures were maintained for 7 days on ascorbate-containing media prior to treatment with either DMSO, AUY922 (100 nM) or BIIB021 (100 nM), every third day (72 h apart). Twenty-four hours after the third treatment, cultures were procured for either biochemical or immunochemical analyses (Rangaraju *et al.*, 2008).

Cell viability assay

[00114] Schwann cells were plated at a seeding density of 10^4 cells/well in a 96-well plate (Nunc), coated with poly-L-lysine (Sigma) and treated either with DMSO or an HSP90 inhibitor at the desired concentrations for 24 h. At the end of the treatment, cells were incubated in a mixture of MTS (3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (333 μ g/ml) and phenazine methosulfate (25 μ M) for 2 h at 37°C, producing the soluble formazan product (Promega). The formazan product was measured spectrophotometrically at 490 nm and graphed as percent of DMSO-treated controls using GraphPad Prism v5.0 software.

Quantitative RT-PCR

[00115] Rat Schwann cells, treated with either DMSO or the selected HSP90 inhibitor compounds (100 nM) were harvested in TRIzol (Invitrogen) and RNA was isolated as per the manufacturer's instructions. One microgram of total RNA was used to synthesize cDNA using 6 the SuperScript III first strand synthesis kit (Invitrogen). The same volume of undiluted cDNA from each sample was used for real time (RT) PCR analysis, using the SYBR GreenER qPCR kit (Invitrogen) and QuantiTect Primer for HSP70 (QT00370489) or GAPDH (QT00199633). The normalized transcript levels of HSP70 relative to geldanamycin (GA) were determined using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). Values obtained were analyzed and graphed with the help of GraphPad Prism v5.0 software.

HSP90 inhibitor administration and rotarod testing

[00116] During the course of the study, the body weight of each mouse was recorded twice/week. Baseline rotarod measurements were obtained on each mouse before the start of the compound treatment (at 8 weeks age). The mice were trained the first two days at 5 rpm

for 60 sec for three trials/day, with 30 min breaks (Madorsky *et al.*, 2009). On the third day, mice were tested on the rotarod, accelerating from 16 rpm to 36 rpm in steps of 4 rpm increase/min (Okamoto *et al.*, 2013). The control group of animals were injected intraperitoneally (i.p.) twice/week with the vehicle consisting of 10% DMSO, 5% Tween-20 and 85% saline (Eccles *et al.*, 2008). The treatment groups received 2 mg/kg AUY922, using the same route and vehicle for administration. Dosage for AUY922 was determined based on the half-life of the compound in plasma (Eccles *et al.*, 2008) and results of the in vitro experiments (see FIGURES. 2-3).

[00117] Rotarod testing was done on all groups simultaneously, once every 2 weeks where all mice underwent the same 3-day rotarod procedure. The time on the rotarod before falling was recorded for each mouse and graphed. The study was terminated at 18 weeks (26 weeks of age) due to some hair loss at the site of injection, although no body weight loss was observed.

***In situ* isometric twitch torque analysis**

[00118] Isometric twitch torque analysis was performed on the tibialis anterior (TA) muscle and anterior tibial tendon. Under anesthesia, the skin and fascia surrounding the distal hindlimb were surgically removed exposing the TA. Braided (4-0) silk surgical suture (Teleflex medical) was secured around the anterior tibial tendon before all tendons to the foot were detached. Mice were positioned in dorsal recumbency on a pre-heated physiology table to maintain body temperature at 37 °C. A clamp was used to secure the hindlimb at 90° at the knee and the paw was positioned on the physiology table using transpore surgical tape (3M). The anterior tibial tendon was secured to a 300C-LR-FP muscle lever (Aurora Scientific). Cathode and anode electrodes were inserted distal to the fibular to stimulate the peroneal nerve. Under control of the Dynamic Muscle Control (DMC) and Analysis (DMA) Software suite (Aurora Scientific) optimal electrode placement was determined by repositioning of the electrodes and stimulating the nerve at 1Hz until maximum twitch amplitude was recorded for a given position. Optimal length-tension was determined by performing isometric twitch stimulation (2500 Hz) at an increasing range of amplitude and tensions until maximum twitch amplitude was observed. Three successive tetanic stimulations (200 Hz, 100 pulses per train, 60 s between independent stimuli) were performed and the muscle was allowed resting for 5 minutes. Single stimulations at 15 Hz, 30 Hz, 60 Hz, 100 Hz, 120 Hz, 160 Hz and 200 Hz were then performed with 30 s between each successive frequency and the resulting torque was recorded and analyzed using DMC and DMA (Aurora Scientific).

[00119] Significance was determined using 2-way ANOVA with Sidak's multiple comparison between individual groups and frequencies. All values are reported as mean $\pm$ S.E.M.

Western blot analyses

[00120] Cell harvesting and tissue homogenization was done in Sodium dodecyl sulfate (SDS) gel sample buffer (62.5 mM Tris pH 6.8, 10% glycerol, 3% SDS), supplemented with protease and phosphatase inhibitors (Rangaraju *et al.*, 2008). Protein concentrations were measured using BCA assay (Pierce). Equal amounts of proteins for each experiment were separated on denaturing SDS gels and transferred to nitrocellulose membrane (0.45 μ m pore size, Bio-Rad). Membranes were blocked in 5% milk (in Tris-buffered saline with 0.05% Tween-20) and incubated with the indicated primary antibodies (Table 1) overnight at 4°C. Bound antibodies were detected with anti-rabbit, anti-goat or anti-chicken HRP-linked secondary antibodies (Sigma) and visualized with the chemiluminescence detection method (Perkin-Elmer Life Sciences). Films were digitally imaged using a GS-800 densitometer (Bio-Rad Laboratories) and were formatted for printing, using Adobe Photoshop 5.5. *Table E1* lists antigens used in the Western blot or immunostaining experiments.

Table E1. Antigens used in the examples.

Species	Antigen	Source and Catalog#	Dilution	
			WB	IS
Rabbit	HSP70	Stressgen; SPA-812	1:3000	n/a
Goat	HSP20	Santa Cruz Biotechnology, Inc.; sc-1049	1:1000	n/a
Mouse	GAPDH	Encor Biotechnology Inc.; MCA-1D4	1:10000	n/a
Mouse	Tubulin	Sigma, St Louis, MO, USA ; T6199	1:2000	n/a
Chicken	P0	Encor Biotechnology Inc.	1:500	n/a
Rat	MBP	Chemicon; MAB386	n/a	1:500

Note: WB means Western Blotting. IS means Immunostaining. n/a means not applicable

Immunostaining

[00121] Explant cultures were fixed in 4% paraformaldehyde (EMS) and permeabilized in 100% ice-cold methanol (Fisher Scientific). After blocking with 5% normal goat serum, primary antibodies (Table 1) were allowed to bind overnight at 4 °C. Bound

antibodies were detected with Alexa Fluor 488 goat anti-rat IgG (Molecular Probes).

Coverslips were mounted using the Prolong Antifade kit (Molecular Probes).

[00122] Proximal region of sciatic nerves were sectioned (5 μ m thickness) and processed as described earlier (Chittoor *et al.*, 2013). Fixed sections were probed with anti-rat and anti-human PMP22 antibodies (Chittoor *et al.*, 2013) in 10% normal goat serum overnight at 4°C. AlexaFluor 594-conjugated goat anti-rabbit antibodies were used to detect the bound primary antibodies. Samples which were processed in parallel without incubation with primary antibodies served as the negative controls. Images were obtained using a SPOT digital camera (Diagnostic Instrumentals, Sterling Heights, MI), with a Nikon Eclipse E800 or an Olympus DSU spinning disc confocal (Tokyo, Japan) microscope, using the same exposure settings. Images were processed using Photoshop 5.5 (Adobe Systems).

Myelin internode length measurement

[00123] DRG cultures were stained for MBP as mentioned above, to label internode segments. The MBP-positive internodes were measured using ImageJ software (NIH). Measurements from three independent experiments, per treatment per genotype, were graphed using GraphPad Prism v5.0 software.

Morphometric analyses of sciatic nerves

[00124] Proximal ends of sciatic nerves from vehicle and AUY922-treated groups were fixed by immersion in ice-cold 2% paraformaldehyde+ 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (Notterpek *et al.*, 1997), at 4 °C overnight. Plastic sections, stained with toluidine blue were obtained from RPAIEMC (Robert P. Apkarian integrated electron microscopy core, Emory). These sections were studied under a light microscope (Zeiss Axioscop 2 plus) and evaluated for axon diameter, fiber diameter (n=950-1100) and total area occupied by nerve fibers (n=20-25) using ImageJ software (NIH) (Madorsky *et al.*, 2009). G-ratio was calculated as axon diameter/ fiber diameter, using the respective values.

Data analyses

[00125] For all comparisons, mean $\pm$ S.E.M was calculated and statistical differences were determined using unpaired two-tailed Student's *t*-test. *P*-values <0.05 (*), <0.01 (**), <0.001 (***) were considered to be significant. For *in situ* torque analysis, significance was determined using two-way ANOVA with Sidak's multiple comparison between individual groups and frequencies.

*Results***Induction of chaperone expression in non-myelinating Schwann cells by HSP90 inhibitors.**

[00126] Five commercially available HSP90 inhibitors, including AT13387, AUY922, BIIB021, SNX5422 and STA9090, were tested for the viability of rat Schwann cells using MTS assay at 50 and 500 nM. After 24 h treatment, Geldanamycin (GA, 50 nM), a well-known inhibitor of HSP90, was found to significantly decrease cellular viability compared to DMSO (FIG. 1A), which is in agreement with studies showing its high toxicity (Miyata, 2005). Among the five compounds tested, lower doses (50 nM) of AT13387, BIIB021 and STA9090 were well tolerated by Schwann cells while the higher doses (500 nM) significantly decreased cell viability, compared to the DMSO-treated cells. Surprisingly, neither concentration of AUY922 affected cell viability, while both concentrations of SNX5422 significantly decreased the values.

[00127] The efficiency of these compounds in chaperone pathway activation in Schwann cells by measuring chaperone expression was also determined. Since HSP70 levels are prominently increased upon HS pathway induction (Saibil, 2013), HSP70 transcript levels were measured after treatment with each inhibitor at 100 nM for 24 h. It was found that HSP70 mRNA levels were markedly increased after treatment with AUY922 and BIIB021, while AT13387 and STA9090 lacked this prominent effect, as compared to GA (FIG. 1B).

[00128] To characterize the effects of the selected compounds on chaperone levels of Schwann cells, dose- and time-dependent studies were performed (FIG. 2). First, Schwann cells were treated with 25, 50 or 100 nM of either AUY922 or BIIB021 for 24 h and then analyzed for levels of HSP70 and HSP27 (FIG. 2A). Both compounds increased HSP70 levels in a dose-dependent fashion, showing peak expression at 100 nM. However, AUY922 was more effective when increasing the levels of HSP70 and HSP27, even at lower doses, as compared to BIIB021. This corresponds with the higher levels of HSP70 mRNA observed upon AUY922 treatment, as compared to BIIB021 (FIG. 1B). Following this, a time course experiment was performed to assess the effects of the compounds on glial chaperones over time (FIG. 2B). As shown, 100 nM BIIB021 or AUY922 increased HSP70 levels as early as 4 h, with expression peaking between 16-24 hours.

[00129] To study the sustainability of the effects of these compounds on HS pathway, Schwann cells were treated with DMSO or 100 nM AUY922 or BIIB021 for 4 hours (treat), followed by media replacement without the drug (chase) (FIG. 2C). BIIB021 and AUY922

maintained elevated chaperone expression for at least 48 hours after the drug was removed, compared to the DMSO treated controls.

Effect of HSP90 inhibitors on myelin production in explant cultures from neuropathic mice.

[00130] The effect of selected HSP90 inhibitors on myelination of peripheral glia was assessed using DRG cultures, which serve as an *in vitro* myelination assay (Rangaraju *et al.*, 2008). DRG explant cultures, obtained from Wt and C22 embryos, were treated with either DMSO (Ct), AUY922 (A, 100 nM) or BIIB021 (B, 100 nM), based on the results from FIG. 2. The cultures were assessed for HSP70 at the end of the treatment as a measure for HS pathway induction (FIG. 3A). As shown, the levels of this chaperone are elevated in HSP90 inhibitor-treated (A and B) Wt and C22 cultures, as compared to their respective DMSO (Ct) samples. However, the increase in HSP70 levels is greater in AUY922-treated C22 cultures compared to the BIIB021- treated. This agrees with results from FIGS. 1 and 2, showing higher efficiency of AUY922 in inducing the pathway compared to BIIB021. Myelin production was evaluated by the increment in myelin protein zero (P0) levels which constitutes the majority of PNS myelin protein composition (FIG. 3A). A prominent increase in P0 levels was observed in the AUY922-treated C22 explants, as compared to the BIIB021- treated cultures. There was a small increase in P0 levels in Wt cultures treated with AUY922 or BIIB021, compared to the DMSO control.

[00131] Since the disease phenotype originates in Schwann cells (Aguayo *et al.*, 1977), experiments were performed to determine whether the glia or nerve cells in the explant cultures respond to HSP90 inhibitors. To test this, DRG cultures from Wt embryos were subjected to FUdR treatment (FIG. 3B, Schwann cell depleted) which specifically kills the mitotically active Schwann cells, leaving the post-mitotic nerve cells unaffected. The response of explant cultures with and without Schwann cells to AUY922 (more potent compound) was compared (FIG. 3B). As shown, the increase in levels of chaperones is prominent in cultures retaining Schwann cells compared to those with only nerve cells. This proves that AUY922 induces the HS pathway in the glia, which expresses the disease-associated PMP22 protein.

[00132] MBP-positive internode lengths, which is a reliable evaluation for healthy myelin (Rangaraju *et al.*, 2008), were measured and graphed as shown in FIG. 3. Both, AUY922 and BIIB021 increase internode lengths in Wt and C22 DRG cultures (FIG. 3C, D).

However, the increase is noteworthy in AUY922-treated cultures compared to DMSO or BIIB021-treated ones. Representative micrographs of each treatment are shown in FIG. 3E.

Effect of HSP90 inhibitor administration on impairment of neuromuscular performance in symptomatic C22 mice.

[00133] To test the effects of the selected HSP90 inhibitor compound, AUY922, on peripheral myelin and motor performance of neuropathic mice, Wt and C22 littermates were randomly segregated at 8 weeks of age into vehicle and AUY922 treatment cohorts. The Wt group was comprised of 6 males and 6 females; while the C22 group consisted of 8 males and 6 females. Animals were injected with 2 mg/kg AUY922 for 18 weeks, twice a week. As shown (FIG. 4A), the overall weight gain of the animals treated with the drug is similar to those injected with vehicle over the period of the study.

[00134] Effects of AUY922 treatment on the motor performance of Wt and C22 mice were assessed on the accelerating rotarod as described in the Methods section. Since there is no significant effect of gender on rotarod performance for the C57BL/6 strain (Hickey *et al.*, 2012); (Zhang *et al.*, 2014), values for male and female groups were combined. At baseline, there is a significant difference between the Wt and C22 vehicle groups (at 8 weeks) and this difference increases with time at 26 weeks (FIG. 4B, C). This is in agreement with studies that show the progressive nature of this disease with age in C22 models (Verhamme *et al.*, 2011); (Chittoor *et al.*, 2013). At baseline, the vehicle and AUY922 treatment groups within the genotypes do not differ in their latencies to fall (FIG. 4B). With treatment, although the AUY922-treated Wt group performs better than the vehicle cohort, it does not reach statistical significance. However, in C22 neuropathic mice, the AUY922 group performs significantly better than its vehicle group, comparable to the Wt animals (FIG. 4C).

[00135] To look at the effects of AUY922 on the muscle component, *in situ* twitch torque analysis was performed on the tibialis anterior (TA) muscle of the animals, prior to study termination (Huguet *et al.*, 2012). This muscle is innervated by the deep peroneal (fibular) nerve and also the tibial branch of the sciatic nerve, and has been used for standardizing this technique in various muscular dystrophy studies (Dellorusso *et al.*, 2001); (Schertzer *et al.*, 2006); (Huguet *et al.*, 2012). Absolute maximal tetanic force generated by the TA muscle after sequential single stimulations of the peroneal nerve was measured and normalized to the body weight of the animal (FIG. 4F). A significant ~28% increase in force was found in C22 animals treated with AUY922 compared to the vehicle. The muscle force in the AUY922 administered C22 animals were similar to that seen in the Wt group,

suggesting an improvement in muscle strength associated with the treatment. Overall, these results indicate that AUY922 administration in the suggested treatment paradigm is tolerated by the animals and results in the attenuation of the decline in neuromuscular performance of the neuropathic animals, with minimal effect on the Wt cohort.

Bioavailability and bioactivity of HSP90 inhibitor.

[00136] To assess the pharmacokinetics of AUY922 in injected mice, the induction of the chaperone pathway in the liver was examined where it is suggested to be metabolized in mice (Eccles *et al.*, 2008). Accordingly, the levels of HSP70 and HSP27 were studied in the livers of vehicle and AUY922-administered animals (FIG. 5A). There is an increase in the chaperone expression in drug-injected mice (2.2-folds), compared to the vehicle cohort of both genotypes. Similar bioactivity was observed in the target tissue and sciatic nerves, where there is a 1.3-fold increase in the expression of HSP70 and HSP27 with AUY922 administration (FIG. 5B). The greater effect of AUY922 on liver chaperones than the sciatic nerve should be taken into consideration while designing further *in vivo* or clinical studies.

[00137] The trafficking assessed through the endoglycosidase H (EndoH) treatment of the exogenous human PMP22 gene in the sciatic nerves of C22 mice is impaired with age (Chittoor *et al.*, 2013). The reduction in the EndoH-resistant fraction associates with the impaired motor behavior of this animal model (Verhamme *et al.*, 2011). To correlate the AUY922-associated improvement in the neuromuscular performance of the C22 animals with the trafficking of PMP22, sciatic nerves of the cohorts were subjected to similar EndoH treatment (FIG. 5C-D). As shown, the EndoH-resistant fraction of PMP22 does not change in the Wt nerves (83.7 ± 5.7 Vs 80.0 ± 7.3), but there is an AUY922-dependent increase by 10.9 times in this fraction in C22 mice (54.3 ± 1.5 vs. 60.2 ± 1.9). These results together suggest that AUY922 efficiently reaches the target tissue and induces the HS pathway, under the specified conditions. This significantly improves the trafficking of PMP22 in peripheral nerves.

Effect of HSP90 inhibitor on myelinated axons in sciatic nerves of C22 neuropathic mice.

[00138] Histopathological defects in peripheral nerves of the C22 mouse model include repeated demyelination and remyelination of medium to large axons with onion bulbs and signs of acute myelin breakdown along with macrophage infiltration (Huxley *et al.*, 1996); (Huxley *et al.*, 1998). Cross-sectional analyses of sciatic nerves from the C22 vehicle

group revealed these characteristics, when compared to the Wt vehicle group (FIG. 6A). However, the occurrence of these disease-associated morphological features was reduced significantly in C22 mice treated with AUY922, compared to the vehicle cohort. The sciatic nerves from AUY922-C22 animals reveal compact myelin with rare occurrence of onion bulbs, similar to the Wt vehicle group. There were no apparent differences in the histology of nerves from Wt vehicle and AUY922 groups.

[00139] The visible improvements in nerve morphology were further corroborated with morphometric analyses on randomly selected cross-sectional micrographs. The total area occupied by fibers in a randomly assigned $40\ \mu\text{m} \times 40\ \mu\text{m}$ square in AUY922-treated C22 animals increases significantly, as compared to its vehicle group, with no prominent difference between the Wt cohorts (FIG. 6B). Upon comparison of axon and fiber diameters (FIG. 6C, D, E), there is no deviation in the overall values in Wt groups (co-efficient of correlation, $r^2=0.96$ in vehicle and AUY922). However, in the C22 neuropathic animals, we see a clear delineation between the vehicle ($r^2=0.94$) and AUY922 ($r^2=0.96$) groups. A similar pattern is obtained when the g-ratio (axon/fiber diameter) was assessed as a function of axon diameters (FIG. 6F, G, H) in C22 animals ($r^2=0.03$ in vehicle versus $r^2=0.23$ in AUY922). This is in contrast to the similar trend seen in vehicle ($r^2=0.29$) and AUY922-treated ($r^2=0.28$) Wt cohorts. These results show that AUY922 administration to the neuropathic mice retains the myelination of axons and thus the overall peripheral nerve morphology.

Effect of HSP90 inhibitor on the occurrence of PMP22-positive aggregates in sciatic nerves of C22 neuropathic mice.

[00140] Presence of PMP22-positive aggregates in the peripheral nerves is a characteristic of the CMT1A mouse models, which is repeatedly observed in the C22 neuropathic animals (Fortun *et al.*, 2003); (Fortun *et al.*, 2006); (Chittoor *et al.*, 2013). To assess the effects of AUY922 administration on PMP22 aggregates, longitudinal sections of the sciatic nerves were stained with PMP22 antibodies that recognize both human and rat protein (Chittoor *et al.*, 2013) (FIG. 7). Quantification of PMP22-positive aggregates in the nerve sections from six random microscopic fields ($0.1\ \text{mm}^2$) showed a prominently higher count in vehicle-treated C22 animals, compared to Wt mice (FIG. 7A). The frequency of these aggregates is reduced by ~ 1.7 -fold (5.0 ± 0.7 Vs 2.9 ± 0.9 ; $*P<0.05$) upon AUY922 treatment in C22 mice. Examination of the nerves for PMP22 localization revealed uniform distribution in peripheral nerves of Wt animals seen in other reports (Chittoor *et al.*, 2013),

from vehicle and AUY922-treated groups (FIG. 7B, insets). However, in the C22 vehicle group, the staining was more focal and revealed perinuclear PMP22 aggregate-like structures (FIG. 7B, arrows). With AUY922 administration, the PMP22 staining appears uniform, similar to nerves from the Wt animals. These results, in agreement with improved PMP22 trafficking (FIG. 5C, D), suggest that AUY922 can suppress the formation of PMP22-positive aggregates in the neuropathic mice.

Discussion

[00141] AUY922 was shown to improve the neuromuscular performance of C22 animal models. This improvement was accompanied by the preservation of the peripheral nerve morphology and increased trafficking of the exogenous PMP22 protein. CMT1A is a protein aggregation disease associated with misexpression of PMP22, largely affecting the motor behavior (Lupski and Garcia, 1992). Despite the availability of animal models and advancement in the understanding of disease nature, very limited therapeutic options are available for CMT1A. C22, a widely used PMP22-overexpression mouse model, represents the most common cause of CMT1A in humans and replicates most of the clinical phenotypic features (Huxley *et al.*, 1996). One of the promising therapeutic approaches, ascorbic acid, displayed prominent improvement of the phenotype in this mouse model (Passage *et al.*, 2004). However, so far it has failed in independently conducted clinical trials (Pareyson *et al.*, 2011); (Lewis *et al.*, 2013). Progesterone antagonists is another therapeutic option which showed improvement in motor performance of PMP22 overexpressor rats (Sereda *et al.*, 2003), but the high toxicity of the currently available progesterone antagonists impedes its evaluation in clinical trials (Pareyson and Marchesi, 2009). Curcumin administration has also proven to be beneficial in improving nerve morphology and motor performance of a PMP22-point mutation mouse model, TrJ (Khajavi *et al.*, 2007). Further verdict awaits on clinical trials of curcumin, which has recently been shown to work through HSP70 (Okamoto *et al.*, 2013), for treatment of CMT1A. Lately another study showed that rapamycin, an activator of autophagy, improves the myelin structure of the sciatic nerves without any significant change in the motor performance in TrJ (Nicks *et al.*, 2014). Promising *in vitro* studies showed significant improvement in myelination of DRG cultures from neuropathic mice (Rangaraju *et al.*, 2010).

[00142] The benefits of HS pathway activation have been repeatedly evidenced in protein misfolding diseases of the central nervous system (Hoshino *et al.*, 2011); (Gifondorwa *et al.*, 2012); (Jiang *et al.*, 2013); (Lin *et al.*, 2013); (Kalmar *et al.*, 2014).

However, there have been very few studies where this pathway has been assessed in PMP22-associated disorders. One indirect study showed the improvement seen in nerve morphology and myelination, paralleled with enhanced motor performance of TrJ mice on an intermittent fasting regimen, with concomitant induction of the HS stress pathway (Madorsky *et al.*, 2009). Another more direct correlation was established by Rangaraju *et al.* (2008), showing that EC137, an HSP90 inhibitor, successfully activated the HS pathway in peripheral glia and improved myelination of cultures from neuropathic C22 mice. This study was not backed by any *in vivo* assessment.

[00143] We started with *in vitro* screening of commercially available compounds. Five commercially available compounds- AT13387, AUY922, BIIB021, SNX5422 and STA9090- were studied in the examples described herein. The effect of HSP90 inhibitors on cellular toxicity is a topic of major concern due to their popularity for the anti-proliferative properties. However their use at a lower dose range activates the stress pathways without causing cell death, which is beneficial for misfolding diseases (Westerheide and Morimoto, 2005). Herein lower doses were selected of the four compounds from the MTS assay, excluding SNX5422 which significantly affected the cellular viability even at the lower dose range (FIG. 1A). AUY922 and BIIB021 showed the best efficacy of the 4 selected drugs to activate the HS pathway in Schwann cells (FIG. 1B). These two compounds were further tested for their effect on *in vitro* myelination by Schwann cells from C22 mice. From these studies, AUY922 was found to be more potent than BIIB021 in increasing myelin synthesis (FIG. 3). Although the reason for this difference is not clear, it could be attributed to higher HSP70 mRNA and protein levels induced by AUY922 compared to BIIB021 (FIG. 1B, 2A, 3D). Since HSP70 individually has been shown to be effective in alleviating protein aggregation in many neurodegenerative diseases (Hoshino *et al.*, 2011); (Gifondorwa *et al.*, 2012); (Jinwal *et al.*, 2013); (Bobkova *et al.*, 2014), this reasoning seems very plausible. This is further supported by the study which showed that the crossing of HSP70-knockout mice with TrJ exacerbated the neuropathic phenotype (Okamoto *et al.*, 2013).

[00144] C22 mice used in examples herein show phenotype within weeks of birth associated with unsteady gait and sudden reaction to loud noises (Huxley *et al.*, 1996). However, they develop distinct motor disabilities, nerve demyelination and muscle atrophy around 6 months of age (Norreel *et al.*, 2003); (Fortun *et al.*, 2006); (Szigeti and Lupski, 2009). Testing herein started at 8 weeks of age, where differences were observed rotarod performance compared to the age-matched Wt mice (FIG. 5B). The mice were injected intraperitoneally with 2 mg/kg of AUY922 twice a week. After 18 weeks of treatment, although

there was no apparent loss in their body weights, the AUY922-injected mice revealed partial loss of hair in the injection area. This was observed in both genotypes and hence the study was terminated at this time. Although the exact reason for this is not known, it may be due to the temporal higher concentration of the drug at the local site of injection. At the end of the study, AUY922 administered C22 animals showed a significantly higher latency time on the accelerating rotarod when compared to the vehicle group. The values of C22-AUY922 mice were closer to the Wt groups, compared to the C22-vehicle (FIG. 5). This is paralleled by preservation of the sciatic nerve morphology in the C22-AUY922 cohort, with myelin thickness and fiber diameter appearing to be similar to the Wt group (FIG. 6). The results suggest that HSP90 inhibitors, particularly AUY922, are potential therapeutic options for CMT1A.

References

1. Aguayo AJ, Attiwell M, Trecarten J, Perkins S, Bray GM (1977) Abnormal myelination in transplanted Trembler mouse Schwann cells. *Nature* **265**:73-75.
2. Ali A, Bharadwaj S, O'Carroll R, Ovsenek N (1998) HSP90 interacts with and regulates the activity of heat shock factor 1 in *Xenopus* oocytes. *Mol Cell Biol* **18**:4949-4960.
3. Bobkova NV, Garbuz DG, Nesterova I, Medvinskaya N, Samokhin A, Alexandrova I, Yashin V, Karpov V, Kukharsky MS, Ninkina NN, Smirnov AA, Nudler E, Evgen'ev M (2014) Therapeutic effect of exogenous hsp70 in mouse models of Alzheimer's disease. *J Alzheimers Dis* **38**:425-435.
4. Chittoor VG, Sooyeon L, Rangaraju S, Nicks JR, Schmidt JT, Madorsky I, Narvaez DC, Notterpek L (2013) Biochemical characterization of protein quality control mechanisms during disease progression in the C22 mouse model of CMT1A. *ASN Neuro* **5**:e00128.
5. Dellorusso C, Crawford RW, Chamberlain JS, Brooks SV (2001) Tibialis anterior muscles in mdx mice are highly susceptible to contraction-induced injury. *J Muscle Res Cell Motil* **22**:467-475.
6. Eccles SA, Massey A, Raynaud FI, Sharp SY, Box G, Valenti M, Patterson L, de Haven Brandon A, Gowan S, Boxall F, Aherne W, Rowlands M, Hayes A, Martins V, Urban F, Boxall K, Prodromou C, Pearl L, James K, Matthews TP, Cheung KM, Kalusa A, Jones K, McDonald E, Barril X, Brough PA, Cansfield JE, Dymock B, Drysdale MJ, Finch H, Howes R, Hubbard RE, Surgenor A, Webb P, Wood M, Wright L, Workman P (2008) NVP-

AUY922: a novel heat shock protein 90 inhibitor active against xenograft tumor growth, angiogenesis, and metastasis. *Cancer Res* **68**:2850-2860.

7. Fortun J, Dunn WA, Jr., Joy S, Li J, Notterpek L (2003) Emerging role for autophagy in the removal of aggresomes in Schwann cells. *J Neurosci* **23**:10672-10680.
8. Fortun J, Go JC, Li J, Amici SA, Dunn WA, Jr., Notterpek L (2006) Alterations in degradative pathways and protein aggregation in a neuropathy model based on PMP22 overexpression. *Neurobiol Dis* **22**:153-164.
9. Gifondorwa DJ, Jimenez-Moreno R, Hayes CD, Rouhani H, Robinson MB, Strupe JL, Caress J, Milligan C (2012) Administration of Recombinant Heat Shock Protein 70 Delays Peripheral Muscle Denervation in the SOD1(G93A) Mouse Model of Amyotrophic Lateral Sclerosis. *Neurol Res Int* **2012**:170426.
10. Hickey MA, Zhu C, Medvedeva V, Lerner RP, Patassini S, Franich NR, Maiti P, Frautschy SA, Zeitlin S, Levine MS, Chesselet MF (2012) Improvement of neuropathology and transcriptional deficits in CAG 140 knock-in mice supports a beneficial effect of dietary curcumin in Huntington's disease. *Mol Neurodegener* **7**:12.
11. Hoshino T, Murao N, Namba T, Takehara M, Adachi H, Katsuno M, Sobue G, Matsushima T, Suzuki T, Mizushima T (2011) Suppression of Alzheimer's disease-related phenotypes by expression of heat shock protein 70 in mice. *J Neurosci* **31**:5225-5234.
12. Huguet A, Medja F, Nicole A, Vignaud A, Guiraud-Dogan C, Ferry A, Decostre V, Hogrel JY, Metzger F, Hoeflich A, Baraibar M, Gomes-Pereira M, Puymirat J, Bassez G, Furling D, Munnich A, Gourdon G (2012) Molecular, physiological, and motor performance defects in DMSXL mice carrying >1,000 CTG repeats from the human DM1 locus. *PLoS Genet* **8**:e1003043.
13. Huxley C, Passage E, Manson A, Putzu G, Figarella-Branger D, Pellissier JF, Fontes M (1996) Construction of a mouse model of Charcot-Marie-Tooth disease type 1A by pronuclear injection of human YAC DNA. *Hum Mol Genet* **5**:563-569.
14. Huxley C, Passage E, Robertson AM, Youl B, Huston S, Manson A, Saberan-Djoniedi D, Figarella-Branger D, Pellissier JF, Thomas PK, Fontes M (1998) Correlation between varying levels of PMP22 expression and the degree of demyelination and reduction in nerve conduction velocity in transgenic mice. *Hum Mol Genet* **7**:449-458.

15. Jiang YQ, Wang XL, Cao XH, Ye ZY, Li L, Cai WQ (2013) Increased heat shock transcription factor 1 in the cerebellum reverses the deficiency of Purkinje cells in Alzheimer's disease. *Brain Res* **1519**:105-111.
16. Jinwal UK, Akoury E, Abisambra JF, O'Leary JC, 3rd, Thompson AD, Blair LJ, Jin Y, Bacon J, Nordhues BA, Cockman M, Zhang J, Li P, Zhang B, Borysov S, Uversky VN, Biernat J, Mandelkow E, Gestwicki JE, Zweckstetter M, Dickey CA (2013) Imbalance of Hsp70 family variants fosters tau accumulation. *Faseb J* **27**:1450-1459.
17. Kalmar B, Lu CH, Greensmith L (2014) The role of heat shock proteins in Amyotrophic Lateral Sclerosis: The therapeutic potential of Arimoclomol. *Pharmacol Ther* **141**:40-54.
18. Khajavi M, Shiga K, Wiszniewski W, He F, Shaw CA, Yan J, Wensel TG, Snipes GJ, Lupski JR (2007) Oral curcumin mitigates the clinical and neuropathologic phenotype of the Trembler-J mouse: a potential therapy for inherited neuropathy. *Am J Hum Genet* **81**:438-453.
19. Lewis RA, McDermott MP, Herrmann DN, Hoke A, Clawson LL, Siskind C, Feely SM, Miller LJ, Barohn RJ, Smith P, Luebke E, Wu X, Shy ME (2013) High-dosage ascorbic acid treatment in Charcot-Marie-Tooth disease type 1A: results of a randomized, doublemasked, controlled trial. *JAMA Neurol* **70**:981-987.
20. Lin PY, Simon SM, Koh WK, Folorunso O, Umbaugh CS, Pierce A (2013) Heat shock factor 1 over-expression protects against exposure of hydrophobic residues on mutant SOD1 and early mortality in a mouse model of amyotrophic lateral sclerosis. *Mol Neurodegener* **8**:43.
21. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) Method. *Methods* **25**:402-408.
22. Lupski JR, Garcia CA (1992) Molecular genetics and neuropathology of Charcot-Marie-Tooth disease type 1A. *Brain Pathol* **2**:337-349.
23. Madorsky I, Opalach K, Waber A, Verrier JD, Solmo C, Foster T, Dunn WA, Jr., Notterpek L (2009) Intermittent fasting alleviates the neuropathic phenotype in a mouse model of Charcot-Marie-Tooth disease. *Neurobiol Dis* **34**:146-154.

24. Mattoo RU, Sharma SK, Priya S, Finka A, Goloubinoff P (2013) Hsp110 is a bona fide chaperone using ATP to unfold stable misfolded polypeptides and reciprocally collaborate with Hsp70 to solubilize protein aggregates. *J Biol Chem* **288**:21399-21411.
25. Miyata Y (2005) Hsp90 inhibitor geldanamycin and its derivatives as novel cancer chemotherapeutic agents. *Curr Pharm Des* **11**:1131-1138.
26. Nicks J, Lee S, Harris A, Falk DJ, Todd AG, Arredondo K, Dunn WA, Jr., Notterpek L (2014) Rapamycin improves peripheral nerve myelination while it fails to benefit neuromuscular performance in neuropathic mice. *Neurobiol Dis* **70**:224-236.
27. Norreel JC, Vinay L, Fontes M, Clarac F (2003) Close relationship between motor impairments and loss of functional motoneuron cells in a Charcot-Marie-Tooth type 1A model. *Neuroscience* **116**:695-703.
28. Notterpek L, Shooter EM, Snipes GJ (1997) Upregulation of the endosomal-lysosomal pathway in the trembler-J neuropathy. *J Neurosci* **17**:4190-4200.
29. Notterpek L, Ryan MC, Tobler AR, Shooter EM (1999) PMP22 accumulation in aggresomes: implications for CMT1A pathology. *Neurobiol Dis* **6**:450-460.
30. Okamoto Y, Pehlivan D, Wiszniewski W, Beck CR, Snipes GJ, Lupski JR, Khajavi M (2013) Curcumin facilitates a transitory cellular stress response in Trembler-J mice. *Hum Mol Genet* **22**:4698-4705.
31. Opalach K, Rangaraju S, Madorsky I, Leeuwenburgh C, Notterpek L (2010) Lifelong calorie restriction alleviates age-related oxidative damage in peripheral nerves. *Rejuvenation Res* **13**:65-74.
32. Pareyson D, Marchesi C (2009) Diagnosis, natural history, and management of Charcot-Marie-Tooth disease. *Lancet Neurol* **8**:654-667.
33. Pareyson D, Reilly MM, Schenone A, Fabrizi GM, Cavallaro T, Santoro L, Vita G, Quattrone A, Padua L, Gemignani F, Visioli F, Laura M, Radice D, Calabrese D, Hughes RA, Solari A (2011) Ascorbic acid in Charcot-Marie-Tooth disease type 1A (CMT-TRIAAL and CMTTRAUK): a double-blind randomised trial. *Lancet Neurol* **10**:320-328.
34. Passage E, Norreel JC, Noack-Fraissignes P, Sanguedolce V, Pizant J, Thirion X, Robaglia-Schlupp A, Pellissier JF, Fontes M (2004) Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. *Nat Med* **10**:396-401.

35. Rangaraju S, Madorsky I, Pileggi JG, Kamal A, Notterpek L (2008) Pharmacological induction of the heat shock response improves myelination in a neuropathic model. *Neurobiol Dis* **32**:105-115.
36. Rangaraju S, Verrier JD, Madorsky I, Nicks J, Dunn WA, Jr., Notterpek L (2010) Rapamycin activates autophagy and improves myelination in explant cultures from neuropathic mice. *J Neurosci* **30**:11388-11397.
37. Saibil H (2013) Chaperone machines for protein folding, unfolding and disaggregation. *Nat Rev Mol Cell Biol* **14**:630-642.
38. Sancho M, Herrera AE, Orzaez M, Perez-Paya E (2014) Inactivation of Apaf1 reduces the formation of mutant huntingtin-dependent aggregates and cell death. *Neuroscience* **262**:83-91.
39. Schertzer JD, Ryall JG, Lynch GS (2006) Systemic administration of IGF-I enhances oxidative status and reduces contraction-induced injury in skeletal muscles of mdx dystrophic mice. *Am J Physiol Endocrinol Metab* **291**:E499-505.
40. Sereda MW, Meyer zu Horste G, Suter U, Uzma N, Nave KA (2003) Therapeutic administration of progesterone antagonist in a model of Charcot-Marie-Tooth disease (CMT-1A). *Nat Med* **9**:1533-1537.
41. Szigeti K, Lupski JR (2009) Charcot-Marie-Tooth disease. *Eur J Hum Genet* **17**:703-710.
42. Urban MJ, Pan P, Farmer KL, Zhao H, Blagg BS, Dobrowsky RT (2012) Modulating molecular chaperones improves sensory fiber recovery and mitochondrial function in diabetic peripheral neuropathy. *Exp Neurol* **235**:388-396.
43. Verhamme C, King RH, ten Asbroek AL, Muddle JR, Nourallah M, Wolterman R, Baas F, van Schaik IN (2011) Myelin and axon pathology in a long-term study of PMP22-overexpressing mice. *J Neuropathol Exp Neurol* **70**:386-398.
44. Westerheide SD, Morimoto RI (2005) Heat shock response modulators as therapeutic tools for diseases of protein conformation. *J Biol Chem* **280**:33097-33100.
45. Zhang Y, Bokov A, Gelfond J, Soto V, Ikeno Y, Hubbard G, Diaz V, Sloane L, Maslin K, Treaster S, Rendon S, van Remmen H, Ward W, Javors M, Richardson A, Austad SN, Fischer K (2014) Rapamycin extends life and health in C57BL/6 mice. *J Gerontol A Biol Sci Med Sci* **69**:119-130.

EQUIVALENTS AND SCOPE

[00145] In the claims articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions of a given product or process that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present, employed, or otherwise relevant, unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments of a given product or process in or to which exactly one member of the group is present, employed, or otherwise relevant. The invention includes embodiments of a given product or process in or to which multiple members of the group are present, employed, or otherwise relevant. The invention includes embodiments of a given product or process in or to which the entire group is present, employed, or otherwise relevant.

[00146] Furthermore, the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims are introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Where elements are presented as lists, *e.g.*, in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, in instances referring to the invention or aspects of the invention as comprising particular elements or features (or both), certain embodiments of the invention or aspects of the invention consist or consist essentially of such elements or features (or both). For purposes of simplicity, those embodiments have not been specifically set forth *in haec verba* herein. It is also noted that the terms “comprising” and “containing” are intended to be open and permit the inclusion of additional elements or steps. Where ranges are given, endpoints are included. Furthermore, unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub-range within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00147] This application refers to various issued patents, published patent applications, journal articles, and other publications, all of which are incorporated herein by reference. If there is a conflict between any of the incorporated references and the instant specification, the specification shall control. In addition, any particular embodiment of the present invention

that falls within the prior art may be explicitly excluded from any one or more of the claims. Because such embodiments are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the invention can be excluded from any claim, for any reason, whether or not related to the existence of prior art.

[00148] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments described herein. The scope of the present embodiments described herein is not intended to be limited to the above Description, but rather is as set forth in the appended claims. Those of ordinary skill in the art will appreciate that various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following claims.

CLAIMS

What is claimed is:

1. A method for treating a neuropathy, the method comprising administering a composition comprising an HSP90 inhibitor to a subject in need thereof.
2. The method of claim 1, wherein the HSP90 inhibitor is BIIB021, or a pharmaceutically acceptable salt thereof.
3. The method of claim 1, wherein the HSP90 inhibitor is AUY922, or a pharmaceutically acceptable salt thereof.
4. The method of claim 1, wherein the HSP90 inhibitor is AT-13387, SNX-5422 (PF-04929113), or STA-9090 (ganetespib), alvespimycin hydrochloride (17-DMAG), ansamycin, elesclomol (STA-4783), gamitrinib, gedunin, geldanamycin, herbimycin A, mabecin I, novobiocin, radicicol, retaspimycin hydrochloride, tanespimycin hydrochloride (17-AAG), 17-GMB-APA-GA, CH518303, CCT018159, CUDC-305, EC144, HSP990, KW-2478, MKT-077, MPC-3100, NMS-E973, NVP-BEP800, PU-29F, PU-H71, TAS-116, TRC-051384, SNX-2112, VER-50589, VER-155008, or XL-888, or a pharmaceutically acceptable salt thereof.
5. The method of any of claims 1-4, wherein the neuropathy is a peripheral demyelinating neuropathy.
6. The method of claim 1-5, wherein the neuropathy is associated with misfolding or aggregation of peripheral myelin protein 22 (PMP22).
7. The method of any of claims 1-6, wherein the neuropathy is a hereditary neuropathy.
8. The method of any of claims 1-7, wherein the neuropathy is anti-MAG peripheral neuropathy, chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain-Barré

syndrome, hereditary neuropathy with liability to pressure palsy (HNPP), or progressive inflammatory neuropathy.

9. The method of any of claims 1-7, wherein the neuropathy is Charcot-Marie-Tooth disease (CMT), Dejerine-Sottas disease, congenital hypomyelinating neuropathy (CHN), Russe-type hereditary motor and sensor neuropathy (HMSNR), CMT with pyramidal features, CMT with optic atrophy, Cowchock syndrome, Rosenberg-Chutorian syndrome, or Roussy- Lévy syndrome.

10. The method of claim 9, wherein the neuropathy is Charcot-Marie-Tooth disease subtype 1A (CMT1A).

11. The method of any of claims 1-6, wherein the neuropathy is caused by diabetes or impaired glucose tolerance.

12. The method of any of claims 1-6, wherein the neuropathy is a chemotherapy-induced peripheral neuropathy (CIPN).

13. A pharmaceutical composition comprising an HSP90 inhibitor for use in treating a neuropathy in a subject, and optionally a pharmaceutically acceptable excipient.

14. The composition of claim 13, wherein the HSP90 inhibitor is BIIB021, or a pharmaceutically acceptable salt thereof.

15. The composition of claim 13, wherein the HSP90 inhibitor is AUY922, or a pharmaceutically acceptable salt thereof

16. The composition of claim 13, wherein the HSP90 inhibitor is AT-13387, SNX-5422 (PF-04929113), or STA-9090 (ganetespib), alvespimycin hydrochloride (17-DMAG), ansamycin, elesclomol (STA-4783), gamitrinib, gedunin, geldanamycin, herbimycin A, macbecin I, novobiocin, radicicol, retaspimycin hydrochloride, tanespimycin hydrochloride (17-AAG), 17-GMB-APA-GA, CH518303, CCT018159, CUDC-305, EC144, HSP990, KW-2478, MKT-077, MPC-3100, NMS-E973, NVP-BEP800, PU-29F, PU-H71, TAS-116, TRC-

051384, SNX-2112, VER-50589, VER-155008, or XL-888, or a pharmaceutically acceptable salt thereof.

17. The method of any of claims 13-16, wherein the neuropathy is a peripheral demyelinating neuropathy.

18. The method of any of claims 13-17, wherein the neuropathy is associated with misfolding or aggregation of peripheral myelin protein 22 (PMP22).

19. The method of any of claims 13-18, wherein the neuropathy is a hereditary neuropathy.

20. The method of any of claims 13-19, wherein the neuropathy is anti-MAG peripheral neuropathy, chronic inflammatory demyelinating polyneuropathy (CIDP), Guillain-Barré syndrome, hereditary neuropathy with liability to pressure palsy (HNPP), or progressive inflammatory neuropathy.

21. The method of claim 13-19, wherein the neuropathy is Charcot-Marie-Tooth disease (CMT), Dejerine-Sottas disease, congenital hypomyelinating neuropathy (CHN), Russe-type hereditary motor and sensor neuropathy (HMSNR), CMT with pyramidal features, CMT with optic atrophy, Cowchock syndrome, Rosenberg-Chutorian syndrome, or Roussy- Lévy syndrome.

22. The method of claim 21, wherein the neuropathy is Charcot-Marie-Tooth disease subtype 1A (CMT1A).

23. The method of any of claims 13-18, wherein the neuropathy is caused by diabetes or impaired glucose tolerance.

24. The method of any of claims 13-18, wherein the neuropathy is a chemotherapy-induced peripheral neuropathy (CIPN).

25. A kit for the treatment of a neuropathy comprising a container comprising a pharmaceutical composition of any of claims 13-23, and instructions for administering the composition to a subject in need thereof.

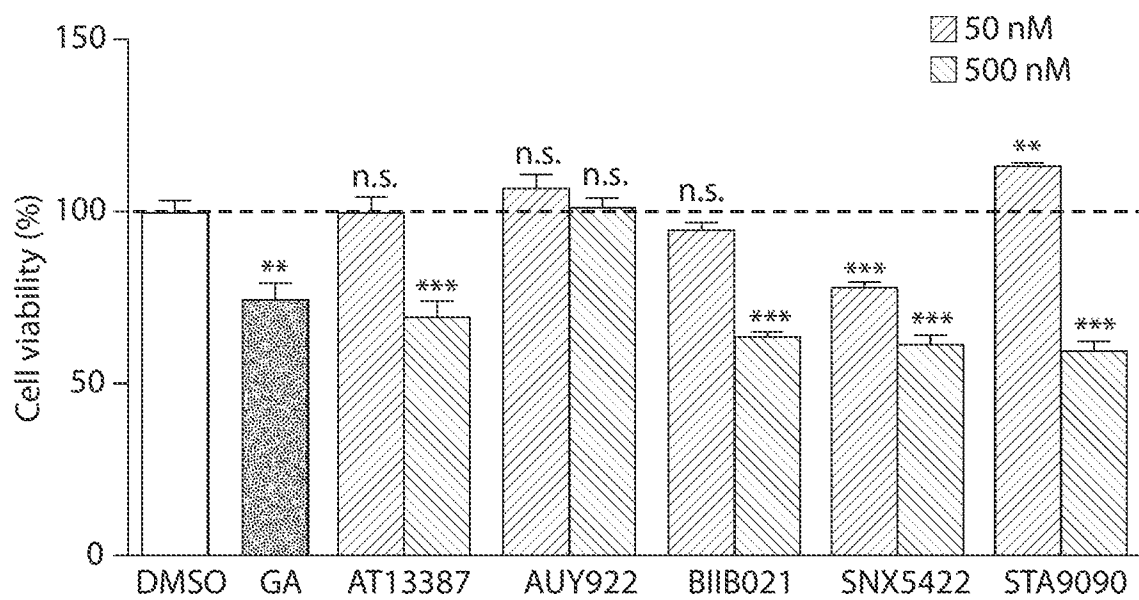


Figure 1A

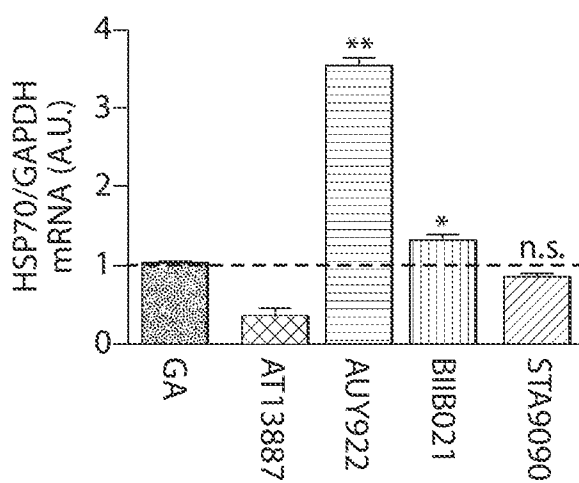


Figure 1B

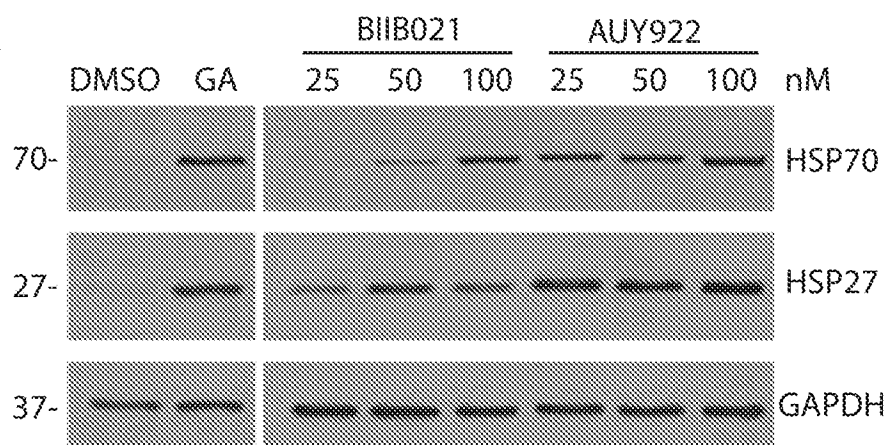


Figure 2A

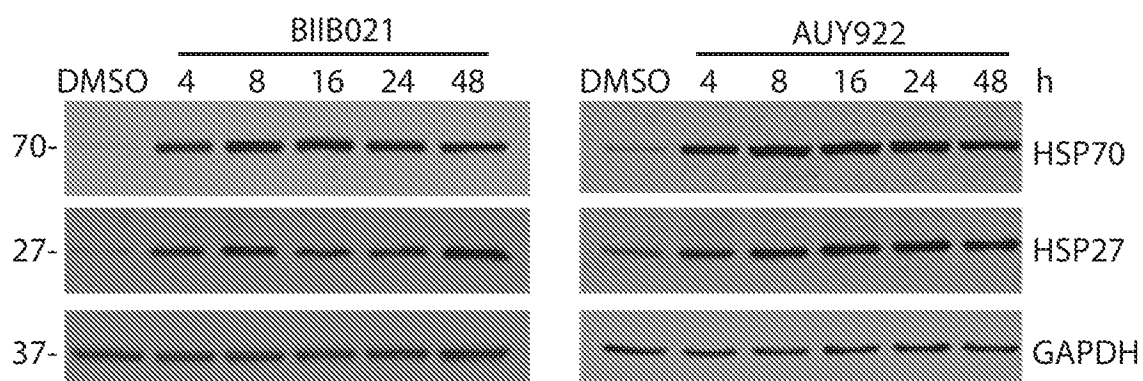


Figure 2B

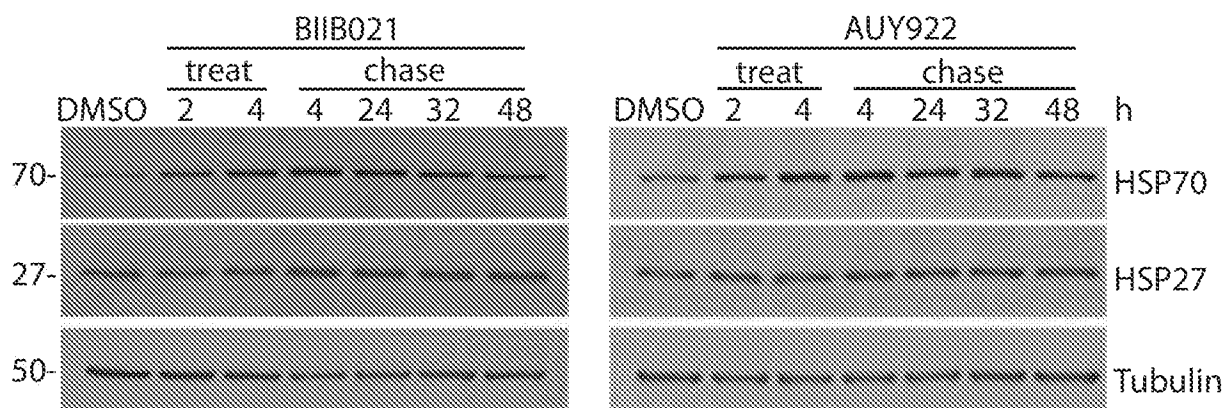


Figure 2C

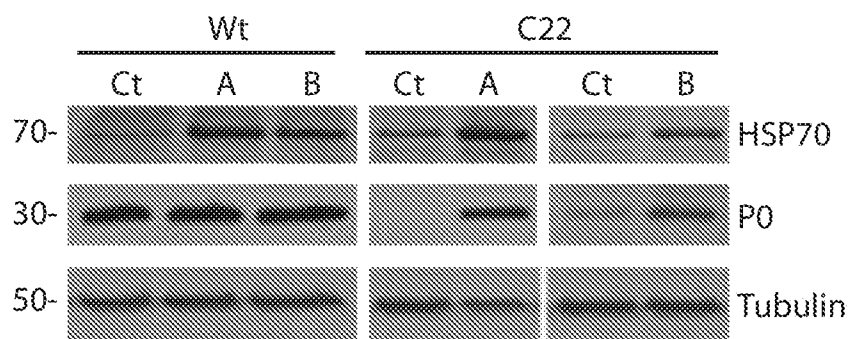


Figure 3A

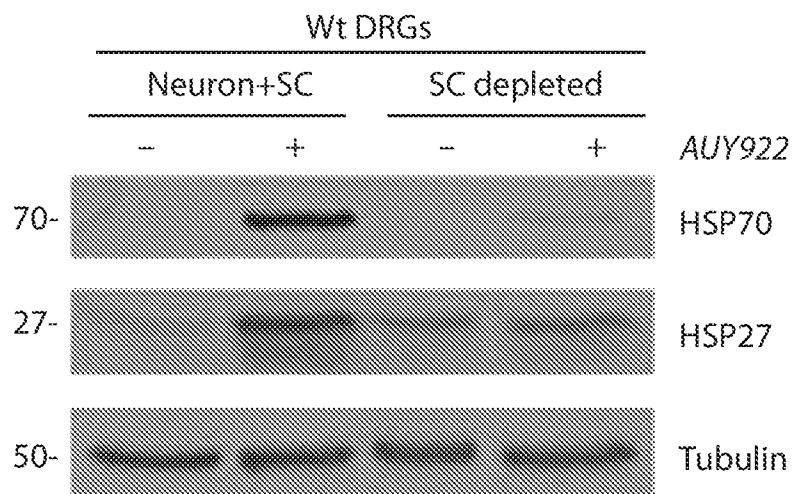


Figure 3B

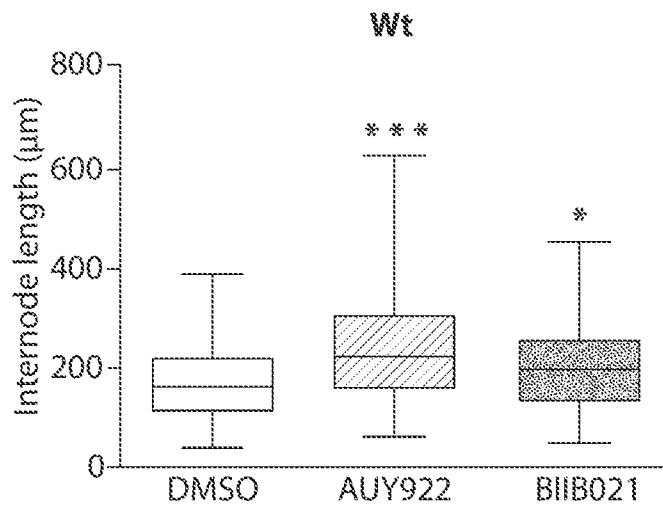


Figure 3C

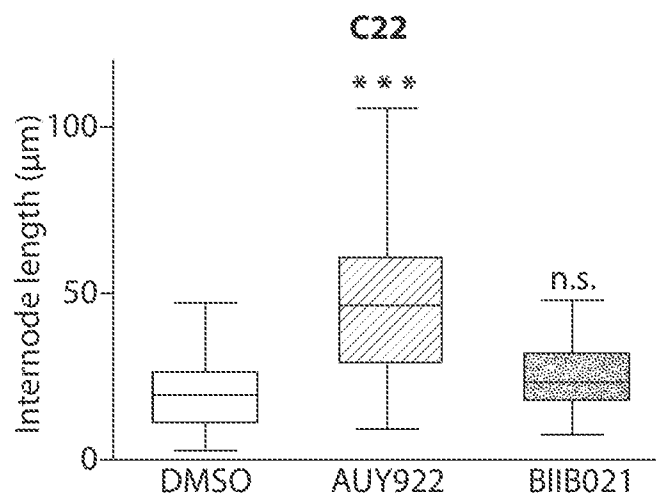


Figure 3D

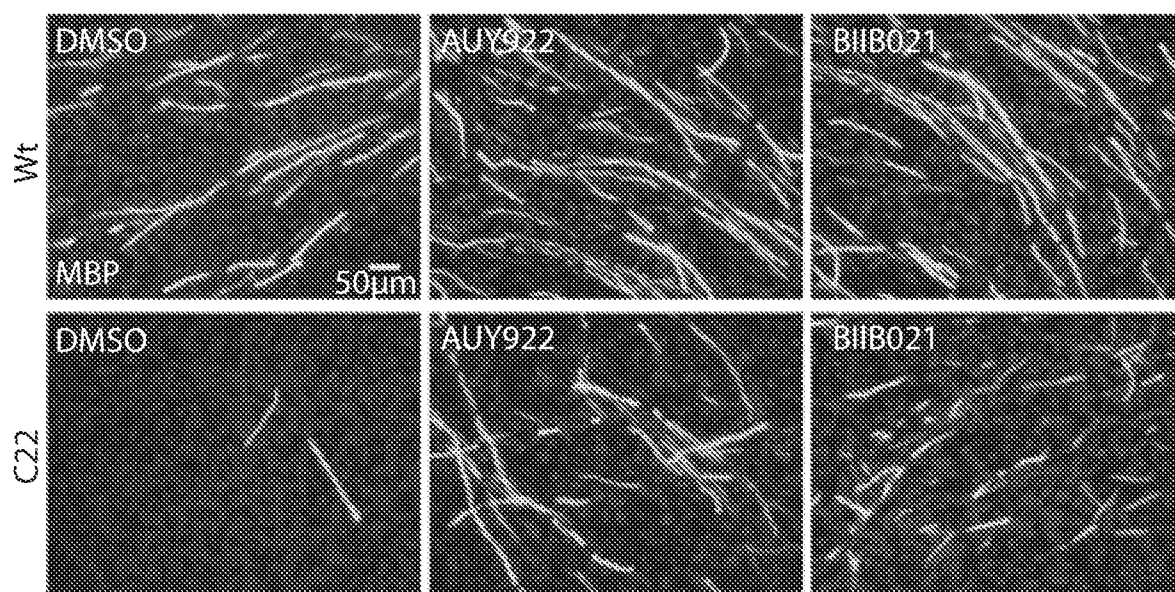


Figure 3E

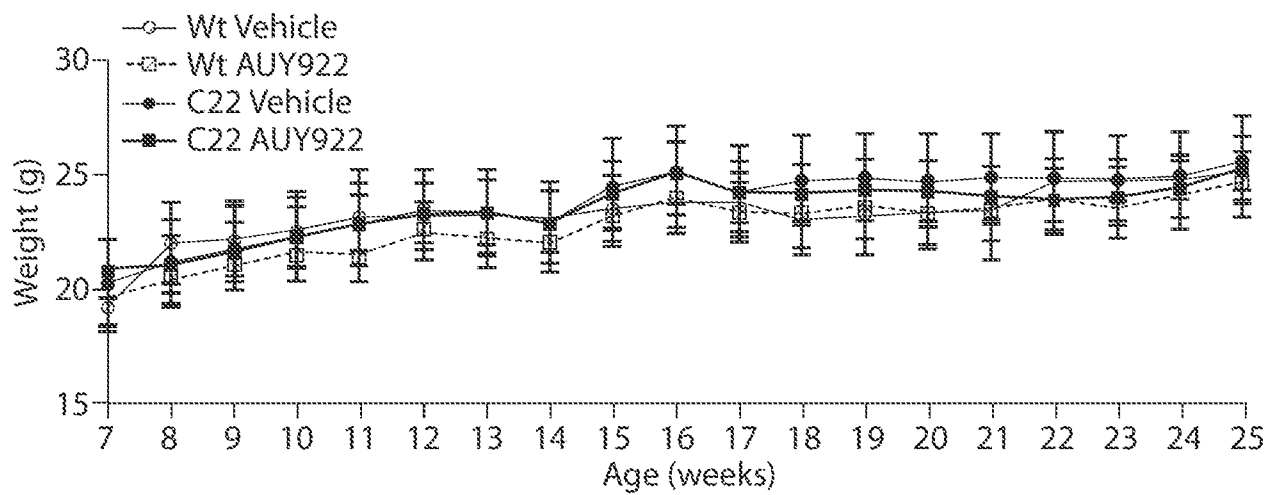


Figure 4A

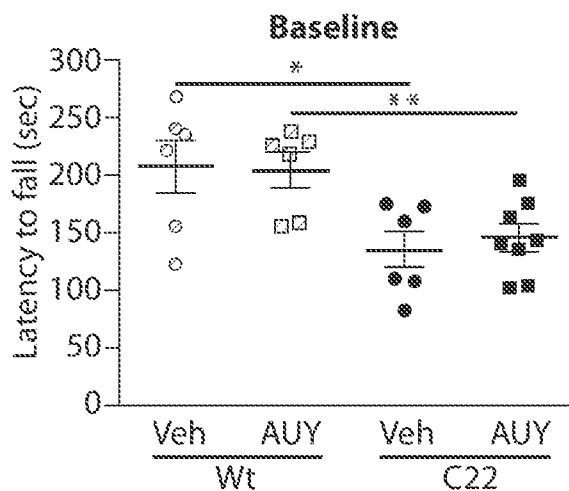


Figure 4B

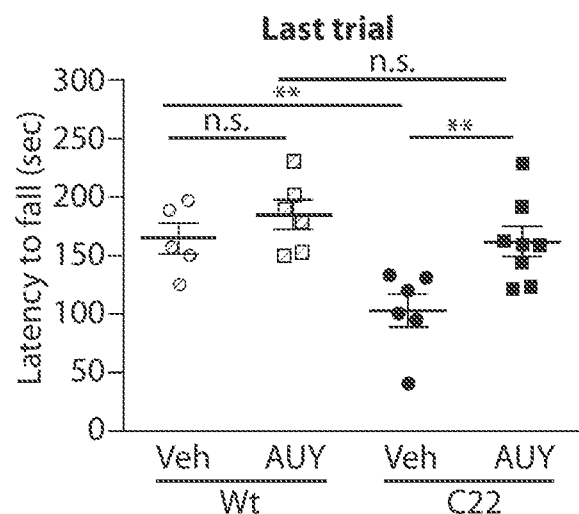


Figure 4C

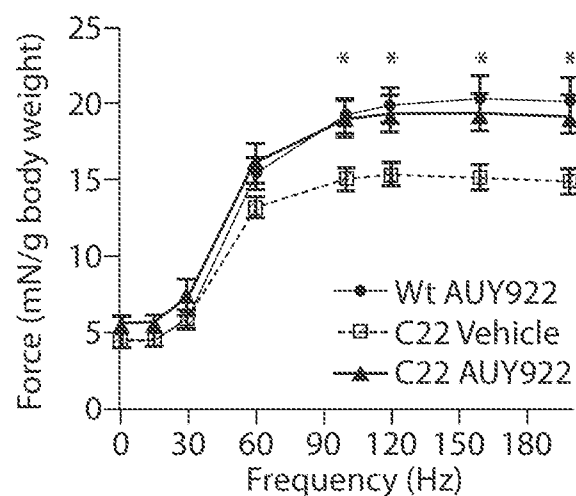


Figure 4D

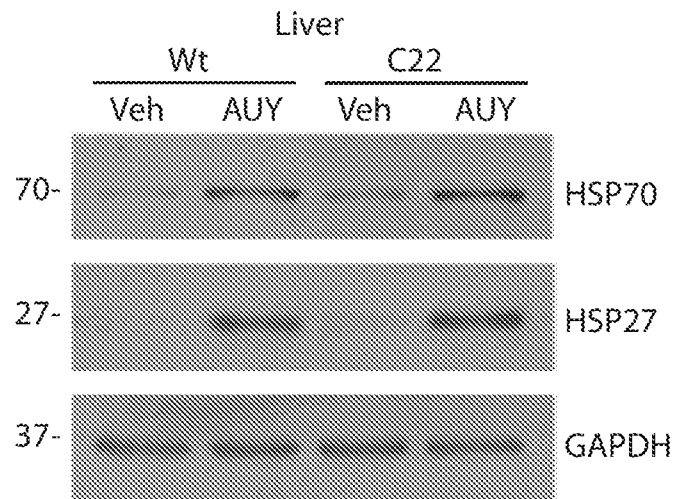


Figure 5A

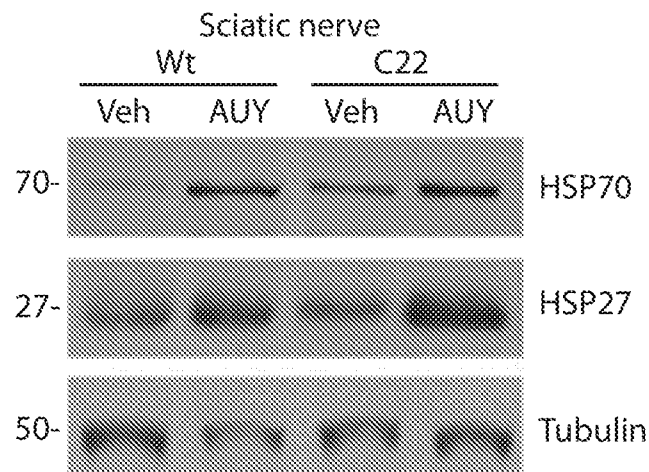


Figure 5B

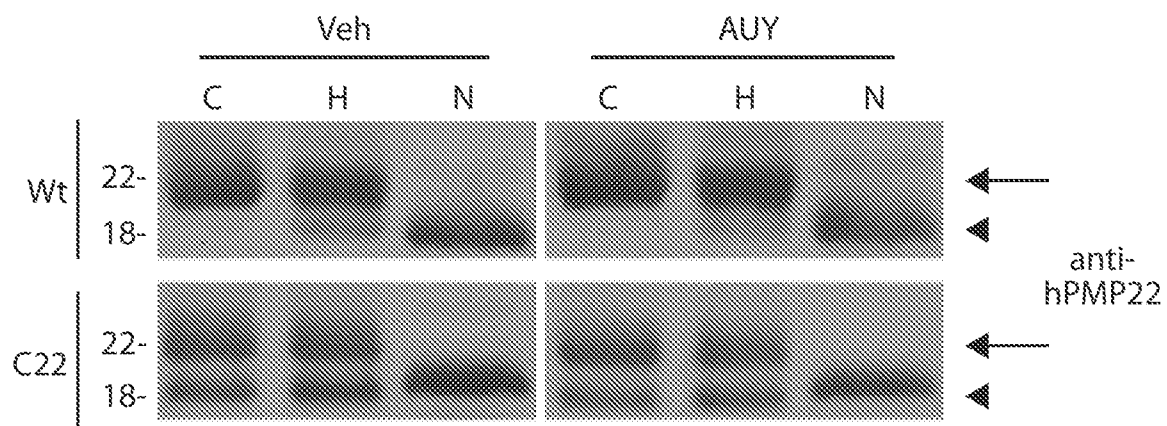


Figure 5C

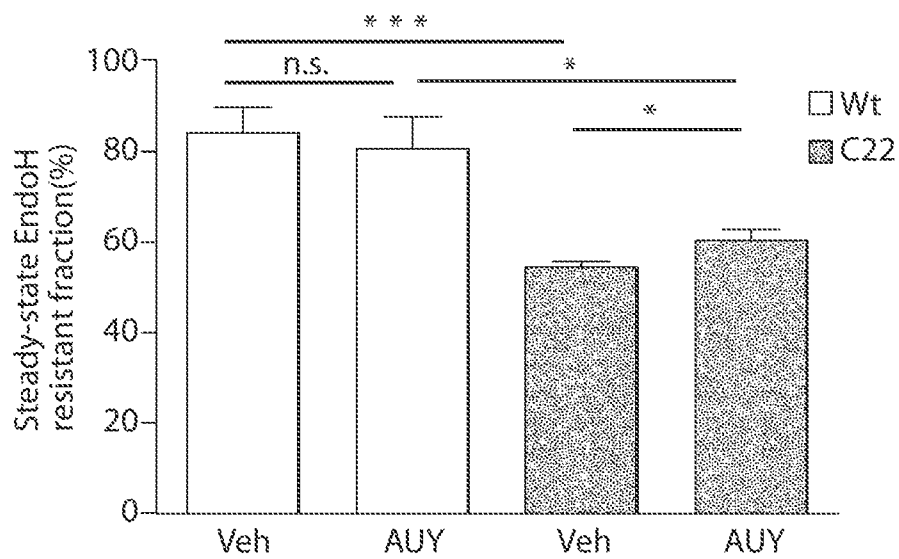


Figure 5D

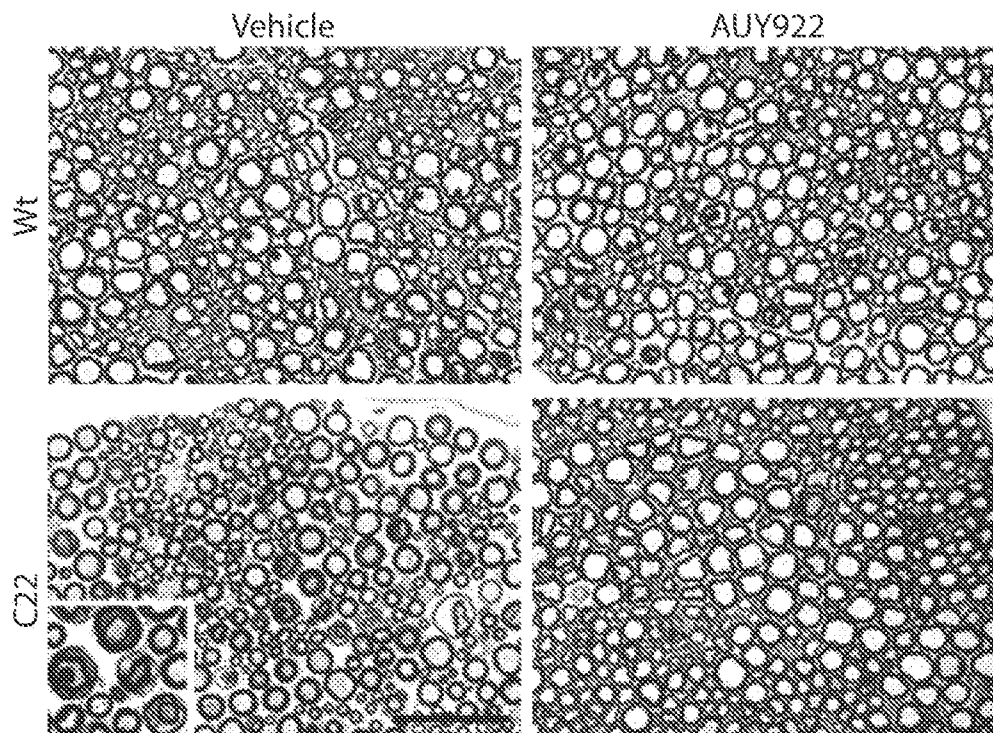


Figure 6A

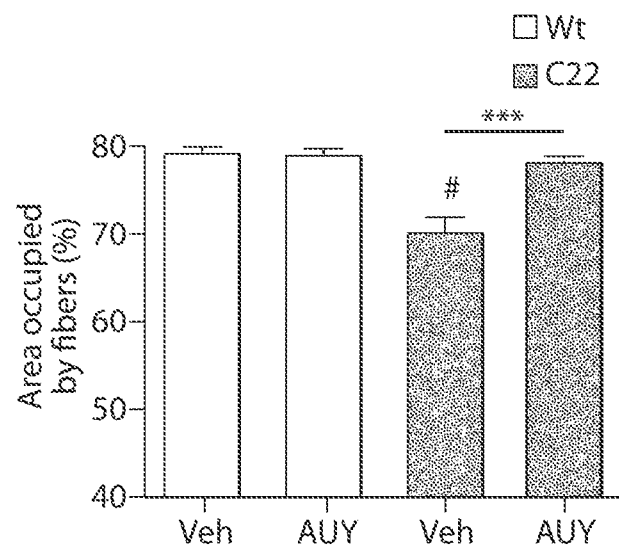


Figure 6B

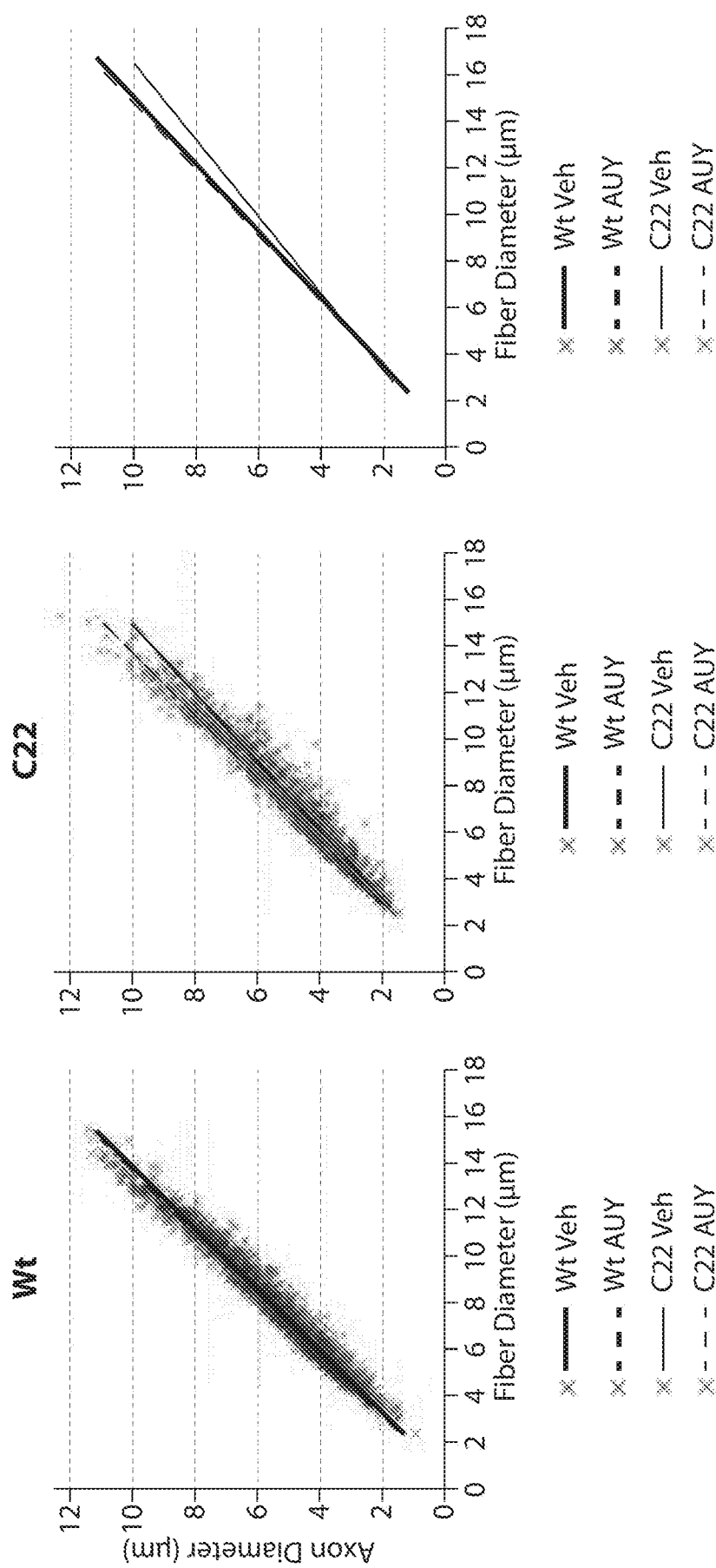


Figure 6E

Figure 6D

Figure 6C

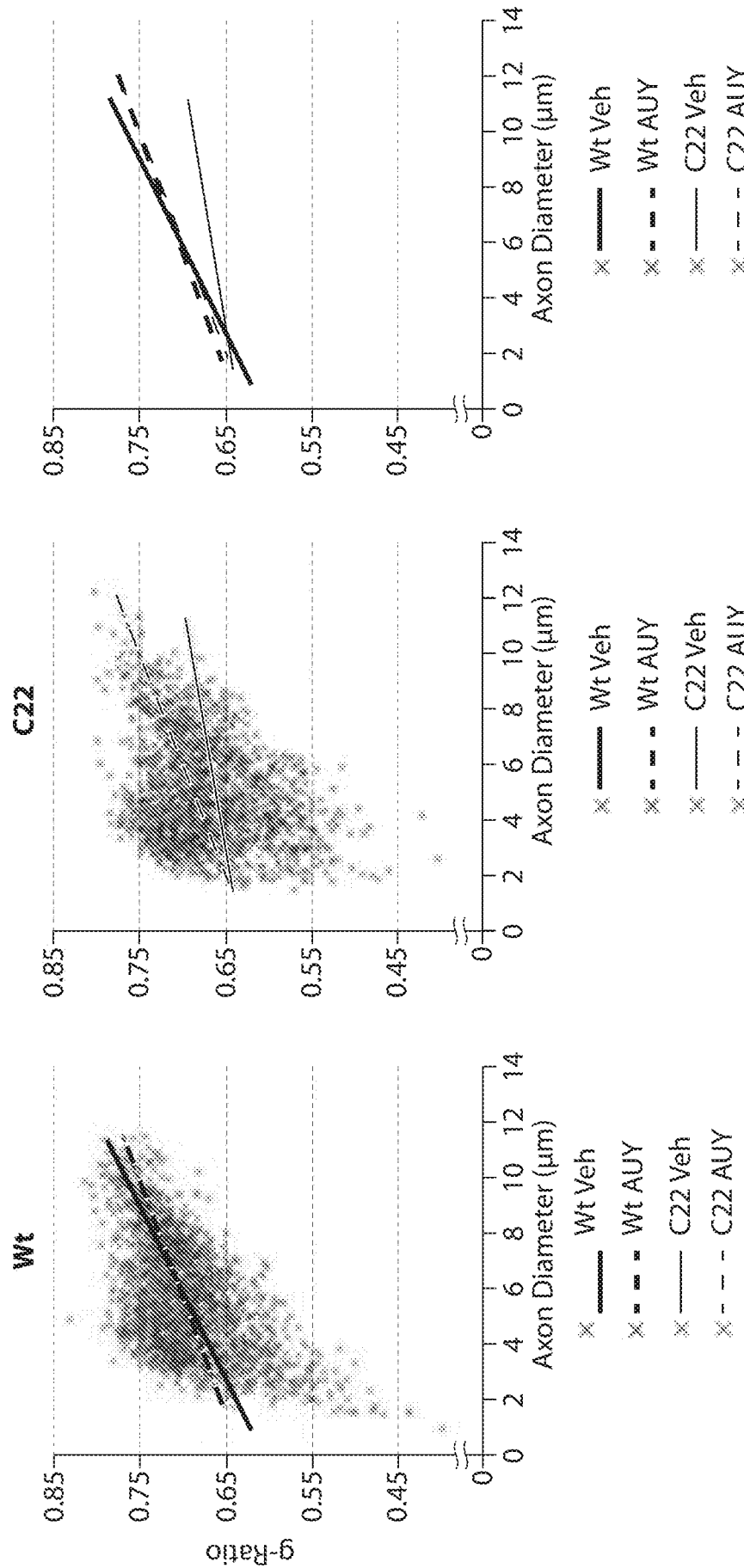


Figure 6H

Figure 6G

Figure 6F

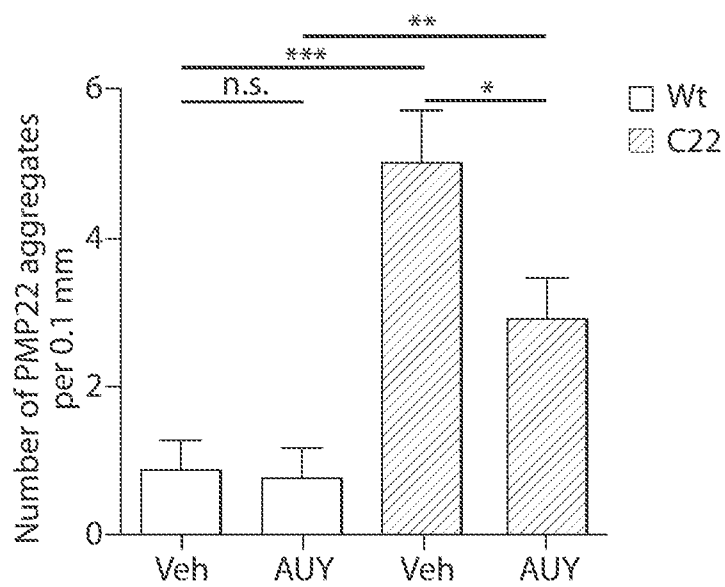


Figure 7A

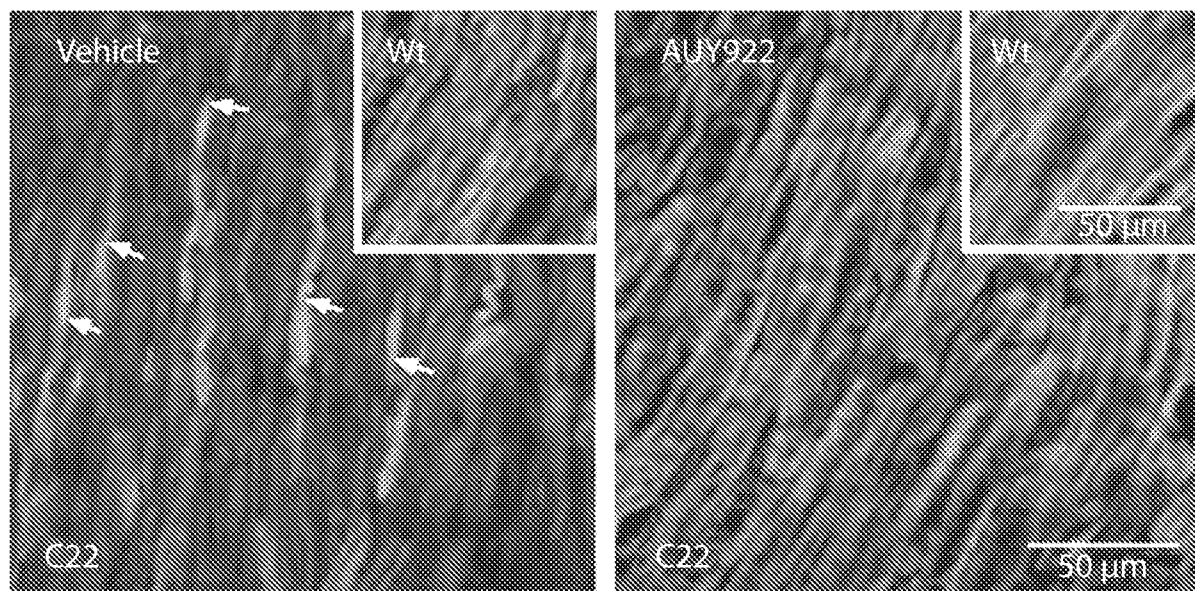


Figure 7B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/21813

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/52; A61P 25/02; C07D 413/04 (2016.01)

CPC - A61K 31/52; C07D 261/02, 413/04

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC (8) - A61K 31/52; A61P 25/02; C07D 261/02, 413/04, 413/10, 473/34, 473/40, 487/02 (2016.01); CPC - A61K 31/52; C07D 261/02, 413/04, 413/10, 473/34, 473/40, 487/02; USPC - 514/263.22, 263.24, 263.4, 378; 544/276

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

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

PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, Other Countries (INPADOC), RU, AT, CH, TH, BR, PH), EBSCO, Google/Google Scholar, PubMed; HSP90, heat shock protein 90, neuropathy, BII021, AUY922, HSP90 inhibitor, AT-13387, ganetespib, SNX-5422, STA-9090, VER-50589, XL-888, peripheral demyelinating neuropathy, Charcot-Marie-Tooth, Chronic inflammatory demyelinating, ansamycin, elesclomol, radicicol, tanespimycin,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----	Rangaraju S et al. "Pharmacological Induction of the Heat Shock Response Improves Myelination in a Neuropathic Model" Neurobiology of Disease October 2008, vol. 32, issue 1, pages 105-115 (pages 1-22); page 1, abstract; page 2, third paragraph; page 8, second paragraph; page 10, first paragraph	1, 5/1 ---
Y		2-4, 5/2-4, 17/13-16
X ----	US 2015/0057240 A1 (UNIVERSITY OF KANSAS) 26 February 2015; paragraphs [0012], [0058]	1, 13 ---
Y		14-16, 17/13-16
Y	TALDONE, T et al. "Targeting Hsp90: Small-Molecule Inhibitors and Their Clinical Development" Current Opinions Pharmacology 2008, vol. 8, no. 4, pages 370-374 (pages 1-7); page 2, second paragraph; page 3, fourth and fifth paragraphs; page 4, first and second paragraphs	2-4, 5/2-4, 14-16, 17/14-16

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

06 May 2016 (06.05.2016)

Date of mailing of the international search report

03 JUN 2016

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US16/21813

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 6-12 and 18-25
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

Remark on Protest

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