(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 November 2013 (14.11.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/169793 A2

(51) International Patent Classification:
A61K 31/519 (2006.01)

(21) International Application Number:
PCT/US2013/039968

(22) International Filing Date:
7 May 2013 (07.05.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/644,937 9 May 2012 (09.05.2012) US

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(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, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, 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, 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, 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, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WO 2013/169793 A2

(54) Title: METHODS AND COMPOSITIONS FOR TDP-43 PROTEINOPATHIES

(57) Abstract: Provided herein are methods for treating a subject suffering from a TDP-43 proteinopathy, e.g., sporadic ALS or FTD by administering to the subject a composition comprising a therapeutically effective amount of a JNK inhibitor, a MAPK inhibitor, a proteasome inhibitor, a Topoisomerase I inhibitor, a Topoisomerase II inhibitor, a HSP-90 inhibitor, a 5-HT antagonist, a CDK inhibitor, or a transcription inhibitor. The provided methods can also be used to reduce TDP-43 aggregation in human neural progenitors or neurons in neural progenitors or neurons that exhibit TDP-43 aggregates. In addition, methods are provided to identify agents that modulate (decrease or increase) TDP-43 aggregation in human neural progenitors or neurons that exhibit TDP-43 aggregates. Also provided are human induced pluripotent stem cell (hiPSC) lines generated from sporadic ALS patients, where the hiPSC lines may be differentiated into neural progenitors or neurons (e.g., motor progenitors and motor neurons) that exhibit a TDP-43 aggregate. Further provided are isolated populations of cells containing neural progenitors or neurons derived from the aforementioned hiPSC lines.

METHODS AND COMPOSITIONS FOR TDP-43 PROTEINOPATHIES

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/644,937, filed May 9, 2012, which application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

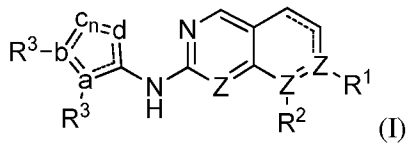
[0001] Proteinopathies are a class of aging-related degenerative disorders characterized by protein misfolding, aggregation, and cellular dysfunction. In particular, TDP-43 aggregates identified in motor neurons of amyotrophic lateral sclerosis (ALS) patients and frontal cortex pyramidal neurons of frontotemporal dementia (FTD) patients, post-mortem, are a hallmark cytopathology for these diseases. Unfortunately, the ability to study the role of endogenous TDP-43 in the pathology of these diseases, and the ability to modulate TDP-43 aggregation in disease-relevant cells has been very limited to date due to the lack of access to live neurons from ALS and FTD patients.

SUMMARY

[0002] Described herein are human induced pluripotent stem cell (hiPSC) lines generated from sporadic ALS patients with the unexpected characteristic that these hiPSC lines can be differentiated in culture into neural progenitors (e.g., motor progenitors) and neurons (e.g., motor neurons) that exhibit endogenous TDP-43 aggregates spontaneously. Also described is the use of such cells for identifying agents that modulate TDP-43 aggregation. Further, the use of compounds of Formula (I) described herein, MAPK, JNK pathway inhibitors for the reduction of TDP-43 aggregation, or for treatment of ALS or FTD is also described.

[0003] Accordingly, in one aspect provided herein is a method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a JNK or a MAPK inhibitor. In some embodiments, the TDP-43 proteinopathy to be treated is ALS (e.g., a sporadic form of ALS) or FTD.

[0004] In another aspect provided herein is a method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a compound having the structure of Formula (I):



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R^1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R^2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

each R^3 is not present or is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, and SO_2R^x;

each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

each Z is selected from C and N;

a is selected from C, N, O, and S;

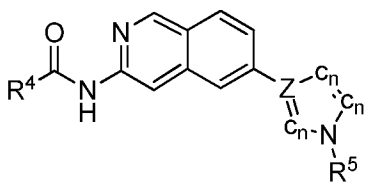
b is selected from C, N, O, and S;

c is selected from C, N, O, and S;

n is zero, one, or 2; and

d is selected from C, N, O, and S, when n is one or 2; or d is selected from O and S, when n is zero.

[0005] In some embodiments, the compound to be administered has the structure of Formula (Ia):



(Ia)

wherein:

R^4 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, and OR^x;

R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

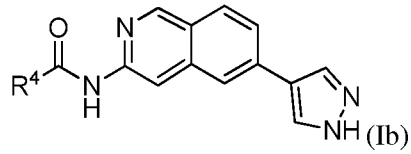
Z is selected from C and N;

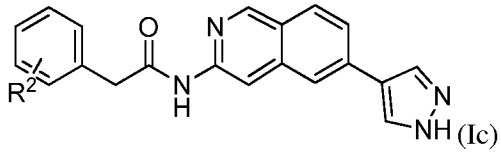
c is selected from C, N, O, and S;

n is zero, one, or 2; and

R^5 is not present or is selected from hydrogen, alkyl, aryl, and heterocyclic.

[0006] In other embodiments, the compound to be administered has the structure of Formula (Ib) or Formula (Ic):


(Ib)


(Ic)

wherein:

R^4 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, and OR^x;

R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl; and

R^2 is selected from alkyl, substituted alkyl, alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino. aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-aryl, and $-SO_2$-heteroaryl.

[0007] In another aspect provided herein is a method for reducing the number or size of TDP-43protein aggregates in a population of cells (e.g., a population comprising neural progenitors, neurons, or both) having TDP-43 protein aggregates with a JNK inhibitor or a compound having the structure of any of Formulas (I), (Ia), (Ib), or (Ic).

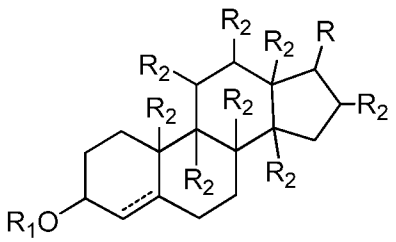
[0008] In a further aspect provided herein is a method for a identifying an agent that modulates (decreases or increases) TDP-43 aggregation in human neurons or human neural progenitors, comprising determining in a population of cells comprising human neurons or human neural progenitors the level of TDP-43 aggregates in the presence or absence of a test agent, wherein a plurality of the human neurons or human neural progenitors contain one or more TDP-43 aggregates in the absence of the test agent; and identifying the test agent as an agent that modulates TDP-43 aggregation in human neurons or human neural progenitors if the TDP-43 aggregation in the plurality of human neurons in the presence of the test agent is different from the TDP-43 aggregation in the plurality of human neurons in the absence of the

test agent. In some embodiments, the plurality of human neurons or human neural progenitors comprises Islet-positive cells. In some embodiments, the plurality of human neurons or human neural progenitors comprises HB9-positive cells. In some embodiments, the plurality of human neurons or human neural progenitors comprises motor neurons. In some embodiments, the population of cells is derived from a subject suffering from ALS (e.g., sporadic ALS) or FTD.

[0009] In another aspect provided herein is an isolated population of human cells comprising viable neurons or neural progenitors, wherein: (i) a plurality of the viable neurons or neural progenitors comprise an endogenous TDP-43 aggregate; and (ii) the isolated population is derived from a subject suffering from ALS or FTD.

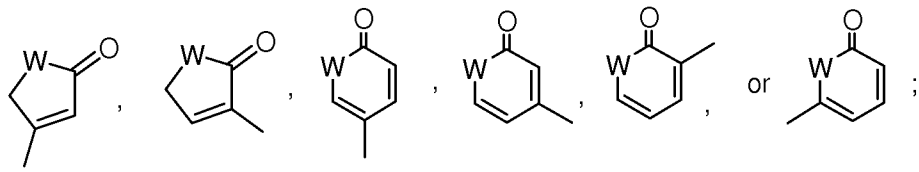
[0010] In another aspect provided herein is a human induced pluripotent stem cell line from a subject suffering from sporadic ALS, wherein a plurality of motor neurons or neural progenitors obtained by differentiating the human induced pluripotent stem cell line comprise a TDP-43 aggregate.

[0011] In another aspect provided herein is a a method for treating a subject suffering from a TDP-43 proteinopathy, which includes administering to the subject a therapeutically effective amount of a compound having the structure of Formula (XIV):



(XIV)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R is  ;

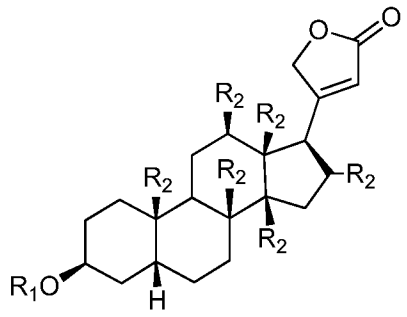
R_1 is selected from a sugar species and hydrogen;

each R_2 is independently selected from OH, alkyl, hydrogen, alkyl, OH, and CHO;

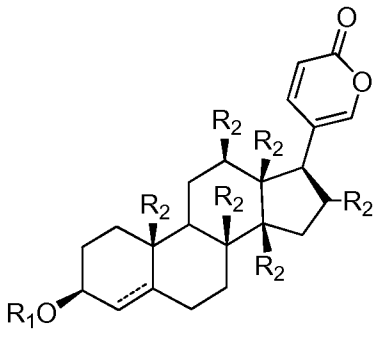
W is selected from O and NR_3; and

R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

[0012] In some embodiments, the structure of Formula (XIV) has the structure of Formula (XIVa) or (XIVb):



(XIVa)



(XIVb)

wherein:

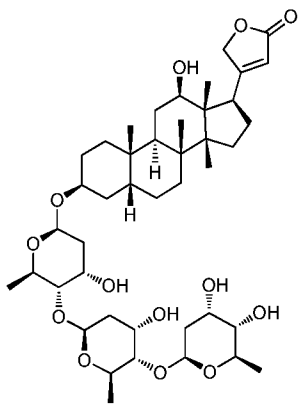
R_1 is selected from a sugar species and hydrogen;

each R_2 is independently selected from OH, alkyl, hydrogen, alkyl, OH, and CHO;

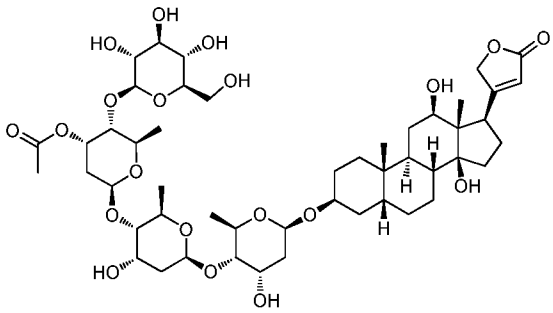
W is selected from O and NR_3; and

R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

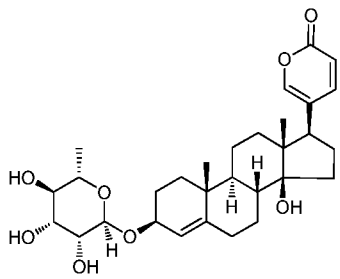
[0013] In other embodiments, the structure of Formula (XIV) has the structure of any of Formulas (XIVc) to (XIVg):



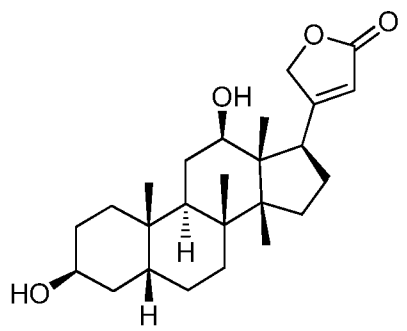
(XIVc)



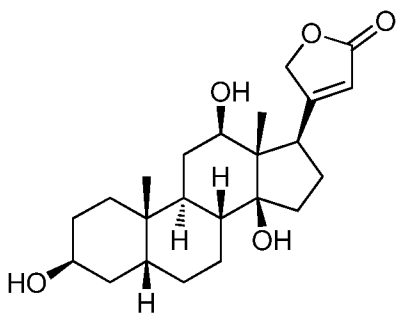
(XIVd)



(XIVe)

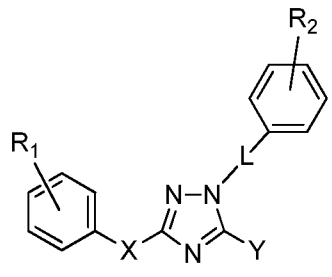


(XIVf)



(XIVg)

[0014] In a further aspect, provided herein is a method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a compound having the structure of Formula (XV):



(XV)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R_1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R_2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

L is not present or is selected from $-C(O)NR_4-$, $-C(O)NR_4$-alkyl-, -C(O)-, $-NR_4-$;

L is not present or is selected from $-C(O)NR_4-$, $-C(O)NR_4$-alkyl-, -C(O)-, $-NR_4-$;

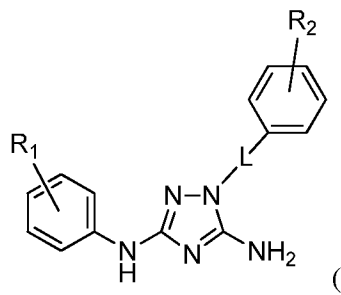
R_4 is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

X is selected from O and NR_3;

R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl; and

Y is selected from OH and NH_2.

[0015] In some embodiments, the structure of Formula (XV) has the structure of Formula (XVa):



(XVa)

wherein:

R_1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R_2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

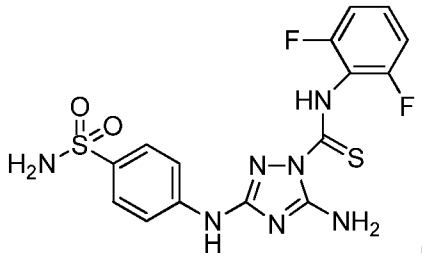
each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

L is not present or is selected from $-C(O)NR_4-$, $-C(O)NR_4$-alkyl-, $-C(O)-$, $-NR_4-$; and

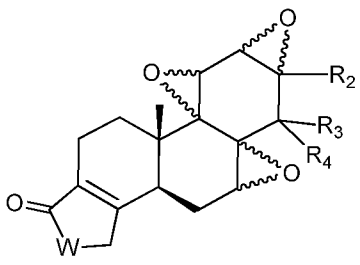
R_4 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

[0016] In other embodiments, the structure of Formula (XV) has the structure of Formula (XVb):



(XVb)

[0017] In yet another aspect, provided herein is a method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a compound having the structure of Formula (XVI):



(XVI)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R^2 is selected from hydrogen, alkyl, alkylhydroxy, hydroxyalkyl, aryl, and heterocyclic;

R^3 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic;

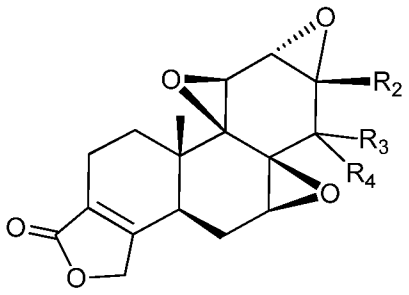
R^4 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic; or

R^3R^4 taken together is =O or NR^1;

W is selected from O and NR^1; and

each R^1 is independently selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic.

[0018] In some embodiments, the structure of Formula (XVI) has the structure of Formula (XVIa):



(XVIa)

wherein:

R^2 is selected from hydrogen, alkyl, alkylhydroxy, hydroxyalkyl, aryl, and heterocyclic;

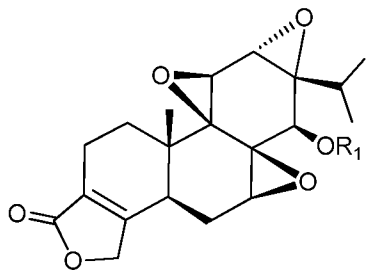
R^3 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic;

R^4 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic; or

R^3R^4 taken together is =O or NR^1; and

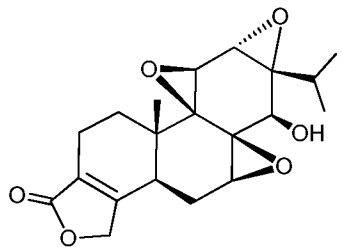
R^1 is selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic.

[0019] In other embodiments, the structure of Formula (XVI) has the structure of Formula (XVIb) or Formula (XVIc):



(XVIb)

wherein R^1 is selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic;



(XVIc)

[0020] In another aspect provided herein is a method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a composition (e.g., a pharmaceutical composition) comprising a therapeutically effective amount of a proteasome inhibitor (e.g., Bortezomib,

MG132, Epoxomicin, or Lactacystin), a Topoisomerase I inhibitor (e.g., Topotecan and Camptothecin), a Topoisomerase II inhibitor (e.g., Doxorubicin and Epirubicin), a heat shock protein 90 (HSP-90) inhibitor (e.g., a) Geldanamycin, 17-AAG (17-N-Allylamino-17-demethoxygeldanamycin), CAY10607), a 5-HT antagonist (e.g., Asenapine), a CDK inhibitor (e.g., Flavopiridol), or a transcription inhibitor (e.g., Actinomycin D).

INCORPORATION BY REFERENCE

[0021] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0023] **Fig. 1** shows Characterization of patient derived iPSC's and iPSC derived motor neurons. A. Phase contrast and immunofluorescence images of control subject and ALS patient derived iPSC's. All iPSC's express the transcription factor NANOG and the surface antigen TRA-1-60. Scale bar = 100 μm. B. Immunofluorescent images of control subject and ALS patient derived motor neurons. Both samples express the motor neuron specific markers ISLET1 (square) and HB9 (circle). Both samples express the neuronal marker βIII-Tubulin. Scale bar = μm.

[0024] **Fig. 2** shows Detailed characterization of patient derived iPSC's. A. Phase contrast and immunofluorescent images of control subject and ALS patient derived iPSC colonies. All samples express the transcription factor NANOG, and the pluripotency related surface antigens SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81. B. Flow cytometry analysis of iPSC's. All samples express the pluripotency related surface antigens SSEA-3, SSEA-4, TRA-1-60, and TRA-1-81. All samples are negative for the mouse specific pluripotency antigen SSEA-1.

[0025] **Fig. 3** Analysis of TDP-43 distribution in relation to other markers. A. Both control and ALS derived motor neurons express TDP-43 and ISLET1. Only ALS derived motor neurons show bright TDP-43 positive aggregates inside the nucleus. B. Costaining of TDP-43 and the nuclear envelope (LAMIN-A). Optical sections were taken in high resolution on a confocal microscope and reconstructed in three dimensions. The image shows clear intranuclear localization of TDP-43 aggregates. C. Automated image

analysis of the distribution of TDP-43 aggregates and ISLET1. Quantitative analysis reveals that only ALS derived motor neurons contain TDP-43 aggregates. In addition, cells positive for ISLET1 are more likely to contain aggregates. D. Immunofluorescence of fibroblasts and iPSC's. Primary fibroblasts and iPSC's derived from ALS patients are negative for TDP-43 aggregates.

[0026] **Fig. 4** A. High magnification image of TDP-43 staining in ALS patient derived motor neurons, showing TDP-43 positive aggregates. B. Costaining of the nuclear envelope marker LAMIN-A and TDP-43. Optical sections were taken with a confocal microscope. Three dimensional reconstruction shows clear nuclear localization of the aggregate.

[0027] **Fig. 5** A. Using grayscale images acquired in all three channels, nuclear masks are identified . B. the TDP-43 channel is used to locate aggregates based on their average intensity, local contrast, and size. The ISLET/HB9 channel is used to distinguish motor neurons from other cell types based on their average nuclear intensity (not shown). C. Information obtained from the previous steps is combined to account only for aggregates within recognized nuclei, and to classify cells as aggregate-positive or aggregate-negative. In addition, the proportion of double positive cells, which are both ISLET/HB9-positive and carry at least one aggregate was also determined.

[0028] **Fig. 6** A small collection of compounds was screened over a dose range in order to identify molecules that modulate the frequency of TDP-43 aggregates. Five active molecules were identified. A. Dose response characteristics of active compounds. The Y-axis denotes the fraction of cells containing TDP-43 aggregates. The X-axis denotes concentration. B. Representative images of fields taken at low and high concentrations of compound. Note the removal of TDP-43 aggregates at high concentrations of the active molecules.

[0029] **Fig. 7** **(A)** shows immunofluorescence staining for TDP-43 in control subject iPSC-cortical neurons (left panel) and sporadic ALS patient iPSC-cortical neurons (right panel). The arrows in the right panel indicate nuclear TDP-43 aggregates. **(B)** immunohistochemistry staining in TDP-43 in post-mortem tissue from a sporadic ALS patient (IPRN.00360) spinal cord (left panel) and temporal lobe (right panel). Arrows in left and right panels denote nuclear TDP-43 aggregates. Arrowhead in left panel denotes cytoplasmic TDP-43 staining.

[0030] **Fig. 8** **(a-c)** shows dose response curve of nuclear TDP-43 aggregation in sporadic ALS patient iPSC-cortical neurons treated with the cardiac glycosides Digoxin (**a**), Lanatoside C (**b**), and Proscillaridin A (**c**); and (**d-f**) dose response of cell viability of sporadic ALS patient iPSC-cortical neurons treated with the same compounds.

DETAILED DESCRIPTION

I. Introduction

[0031] Described herein are hiPSC lines generated from sporadic ALS patients, wherein such hiPSC lines yield, by *in vitro* differentiation, neural progenitors (e.g., motor progenitors) and neurons (e.g., motor neurons) that, unexpectedly, exhibit TDP-43 aggregates. Importantly, only a fraction of sporadic ALS patient-derived hiPSC lines yield such cells. Nevertheless, such hiPSC lines can be identified as described herein. Cultures of TDP-43 aggregate-bearing neural progenitors and neurons are also used to identify agents that can modulate TDP-43 aggregation in such cells. Further described herein is a class of compounds of Formula (I) that decreases TDP-43 aggregates and can be used to treat TDP-43 proteinopathies, e.g., ALS or FTD. Also described are the use of JNK kinase pathway inhibitors to reduce TDP-43 aggregates or for the treatment of TDP-43 proteinopathies, e.g., ALS or FTD

II. Definitions

[0032] "ALS," as used herein, refers to the neurodegenerative condition known as amyotrophic lateral sclerosis (also known as "Lou Gehrig's Disease"). Unless otherwise noted, ALS refers to familial or sporadic form of ALS.

[0033] "effective amount," as used herein, is an amount, which when administered systemically, is sufficient to effect beneficial or desired results, such as beneficial or desired clinical results, or enhanced locomotion, motor coordination, respiration, or other desired effects. An effective amount is also an amount that produces a prophylactic effect, e.g., an amount that delays, reduces, or eliminates the appearance of a pathological or undesired condition. Such conditions include, but are not limited to, ALS, FTD, and chronic traumatic encephalopathy (CTE). An effective amount is optionally administered in one or more administrations. In terms of treatment, an "effective amount" of a composition described herein is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of a TDP-43 proteinopathy, e.g., ALS. An "effective amount" includes any therapeutic agent, e.g., a JNK kinase inhibitor, used alone or in conjunction with one or more agents used to treat a disease or disorder. An "effective amount" of a therapeutic agent as described herein will be determined by a patient's attending physician or other medical care provider. Factors which influence what a therapeutically effective amount will be include, the absorption profile (e.g., its rate of uptake into the CNS or spinal cord) of a therapeutic agent, time elapsed since onset of the TDP-43 proteinopathy, and the age, physical condition, existence of other disease states, and nutritional status of the individual being treated. Additionally, other medication the patient is receiving, e.g., pain medications used in combination

[0034] with a therapeutic agent, will typically affect the determination of the therapeutically effective amount of the therapeutic agent to be administered.

[0035] "induced pluripotent stem cell," as used herein, refers to a pluripotent stem cell derived from a postnatal somatic cell by any combination of forced expression of reprogramming factors alone or in combination with one or more reprogramming agents.

[0036] As used herein the term "isolated," with reference to a cell or a cell population, refers to a cell or cell population that is in an environment different from that in which the cell or cell population naturally occurs. An isolated cell can be present in a mixed population of cells, where the population can be said to be "enriched" for the isolated cell. For example, an isolated iPS cell can be present in a mixed population of cells *in vitro*, where the mixed population comprising iPSCs and cells that are not iPSCs. An "enriched" population of iPSCs is a cell population in which at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 98%, or more than 98%, of the cells in the cell population are iPSCs.

[0037] "inhibitor," as used herein, refers to a molecule which is capable of inhibiting one or more of the biological activities of a target molecule, such as enzyme activity or interaction with a binding partner that modulates enzyme activity.

[0038] "JNK," or "JNK kinase," as used herein, unless otherwise specified, refer to a c-Jun N-terminal kinase isoform. The c-Jun N-terminal kinases consist of ten isoforms derived from three genes: JNK1 (four isoforms), JNK2 (four isoforms) and JNK3 (two isoforms). Each gene is expressed as either 46 kDa or 55 kDa protein kinases, depending upon how the 3' coding region of the corresponding mRNA is processed. A second form of alternative splicing occurs within transcripts of JNK1 and JNK2, yielding JNK1-α, JNK2-α and JNK1-β and JNK2-β.

[0039] "JNK activity," or "MAPK activity," as used herein, unless otherwise specified, includes, but is not limited to, at least one of JNK or MAPK protein-protein interactions, phosphotransferase activity (intermolecular or intermolecular), translocation, etc of one or more isoforms.

[0040] "JNK inhibitor," or "MAPK inhibitor," as used herein, refers to any molecule, compound, or composition that directly or indirectly decreases the JNK or MAPK activity. In some embodiments, JNK inhibitors inhibit, decrease, and/or abolish the level of a JNK mRNA and/or protein or the half-life of JNK mRNA and/or protein, such inhibitors are referred to as "clearance agents". In some embodiments, a JNK inhibitor is a JNK antagonist that inhibits, decreases, and/or abolishes an activity of JNK. In some embodiments, a JNK inhibitor also disrupts, inhibits, or abolishes the interaction between JNK and its natural binding partners or substrates (e.g., JIP1, POSH, ELK1, c-Jun, JunB, JunD, JDP2,and HSF1), a substrate for a JNK or a protein that is a binding partner of JNK in a pathological condition, as measured using standard methods. In some embodiments, JNK inhibitors reduce, abolish, and/or remove the binding between JNK and at least one of its natural binding partners (e.g., HSF1). Thus, binding between JNK and at least one of its natural binding partners is stronger in the absence of the inhibitor than in its presence. Alternatively or additionally, JNK inhibitors inhibit the phosphotransferase activity of JNK,

e.g., by binding directly to the catalytic site or by altering the conformation of JNK such that the catalytic site becomes inaccessible to substrates.

[0041] In some embodiments, JNK inhibitors inhibit the ability of JNK to phosphorylate at least one of its target substrates, e.g., HSF1, JunB, JunD; or itself, i.e., autophosphorylation. JNK inhibitors include inorganic and/or organic compounds.

[0042] In some embodiments, a pharmacological composition comprising a JNK inhibitor is "administered peripherally" or "peripherally administered." As used herein, these terms refer to any form of administration of an agent, e.g., a therapeutic agent, to an individual that is not direct administration to the CNS, i.e., that brings the agent in contact with the non-brain side of the blood-brain barrier. "Peripheral administration," as used herein, includes intravenous, intraarterial, subcutaneous, intramuscular, intraperitoneal, transdermal, by inhalation, transbuccal, intranasal, rectal, oral, parenteral, sublingual, or trans-nasal. In some embodiments, a JNK inhibitor is administered by an intraspinal route.

[0043] "MAPK," as used herein, refers to "mitogen-activated protein kinase."

[0044] "motor progenitor," as used herein, refers to a neural progenitor, which is capable of giving rise to a terminally differentiated motor neuron under appropriate culture conditions.

[0045] "neural progenitor," as used herein, refers to a cell with the property of self-renewal, which is capable of giving rise to a terminally differentiated neuron under appropriate culture conditions.

[0046] "reprogramming factor," as used herein, refers to any gene product, though usually a polypeptide, that alone or in combination with other reprogramming factors or reprogramming agents reprograms a postnatal somatic cell to become a pluripotent stem cell.

[0047] "subject" or an "individual," as used herein, is a human patient or a healthy human. In some embodiments a "subject" or an "individual" is a human. In some embodiments, the subject suffers from ALS or FTD.

[0048] "TDP-43 aggregate," as used herein, refers to a heterogeneous, multimeric, macromolecular complex that is reactive with antibodies against TDP-43, TDP-43 phosphorylation, or any fragments thereof, although such aggregates may also include proteins other than TDP-43 or TDP-43 fragments.

[0049] "TDP-43 Proteinopathy," as used herein, refers to any condition characterized by aberrant localization or aggregation of TDP-43. Examples of TDP-43 proteinopathies include, but are not limited, to ALS (e.g., sporadic ALS), FTD, Chronic Traumatic Encelopathy, Parkinson's Disease, Dementia with Lewy Bodies, Alzheimer's Disease, Pick's Disease, Spino Cerebellar Ataxia 2, Sporadic body inclusion myositis, Hippocampal sclerosis, and progressive supranuclear palsy.

[0050] "Treatment" or "treating," as used herein, includes achieving a therapeutic benefit and/or a prophylactic benefit. By therapeutic benefit is meant eradication or amelioration of the underlying disorder or condition being treated. For example, in an individual with ALS, therapeutic benefit includes partial or

complete halting of the progression of the disorder, or partial or complete reversal of the disorder. Also, a therapeutic benefit is achieved with the eradication or amelioration of one or more of the neurological symptoms associated with the underlying condition such that an improvement is observed in the patient, notwithstanding the fact that the patient is still affected by the condition. A prophylactic benefit of treatment includes prevention of a condition, retarding the progress of a condition, or decreasing the likelihood of occurrence of a condition. As used herein, "treating" or "treatment" includes prophylaxis.

III. Methods

Overview

[0051] The methods described herein are drawn to the treatment of TDP-43 proteinopathies including, but not limited to, ALS and FTD, by administering a therapeutically effective amount of a pharmaceutical composition comprising a JNK inhibitor, a MAPK inhibitor, or a compound having the structure of Formula (I), as described herein. Also described are methods for identifying agents that modulate TDP-43 aggregation in neural progenitors and neurons (e.g., motor progenitors and motor neurons)

Treatment of TDP-43 Proteinopathies

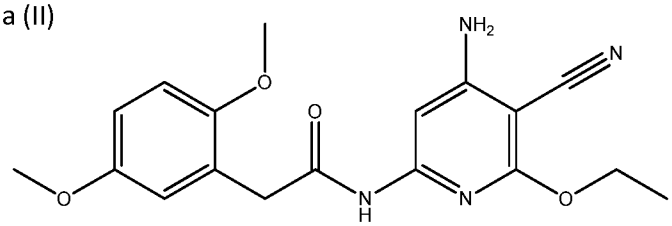
[0052] In some embodiments, the methods described herein are used to treat a subject suffering from a TDP-43 proteinopathy. Examples of TDP-43 proteinopathies include, but are not limited to: ALS, e.g., sporadic ALS or familial ALS. FTD, e.g., frontaltemporal lobar degeneration (FTLD) with ubiquitin inclusions (FTLD-U); FTLD associated with motor neuron disease (FTLD-MND), and Alzheimer's Disease (AD), e.g., AD with with hippocampal sclerosis. In some embodiments, the methods described herein are used to reduce the number or size of TDP-43 aggregates in neural progenitors (e.g., motor progenitors) or neurons (e.g., motor neurons) of patients suffering from any of the above-mentioned conditions. In one embodiment, TDP-43 aggregates are reduced in neural progenitors or neurons in culture, e.g., neurons obtained by differentiation of patient hiPSC lines, or by direct reprogramming of fibroblasts into neurons (Pang *et al* (2011), *Nature*, published online May 26, 2011.). In some embodiments, the subject or cells to be treated are treated with a JNK inhibitor as described herein.

[0053] JNK kinases consist of ten isoforms derived from three genes: JNK1 (four isoforms), JNK2 (four isoforms) and JNK3 (two isoforms). Each gene is expressed as either 46 kDa or 55 kDa protein kinases, depending upon how the 3' coding region of the corresponding mRNA is processed. A second form of alternative splicing occurs within transcripts of JNK1 and JNK2, yielding JNK1-α, JNK2-α and JNK1-β and JNK2-β. JNK1 and JNK2 are found in all cells and tissues. JNK3 is found mainly in the brain, but is also found in the heart and the testes.

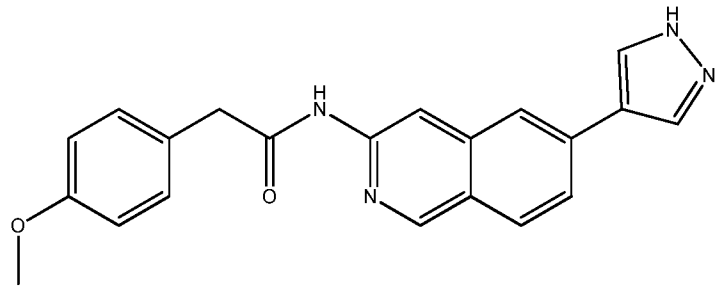
JNK Inhibitors

[0054] Examples of JNK inhibitors suitable for use in the methods described herein include, but are not limited to, any of the following compounds, or pharmaceutically acceptable salts or solvates thereof:

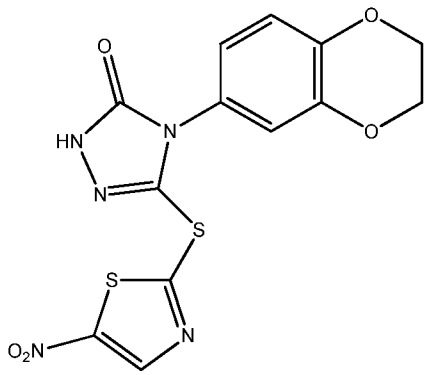
Formula (II)



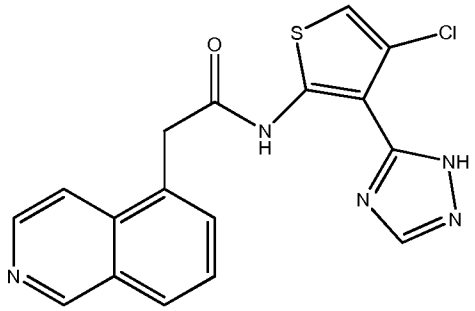
Formula (III)



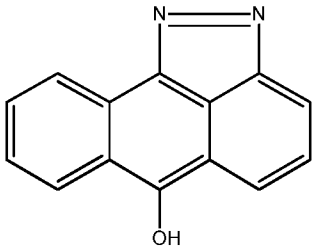
Formula (IV)



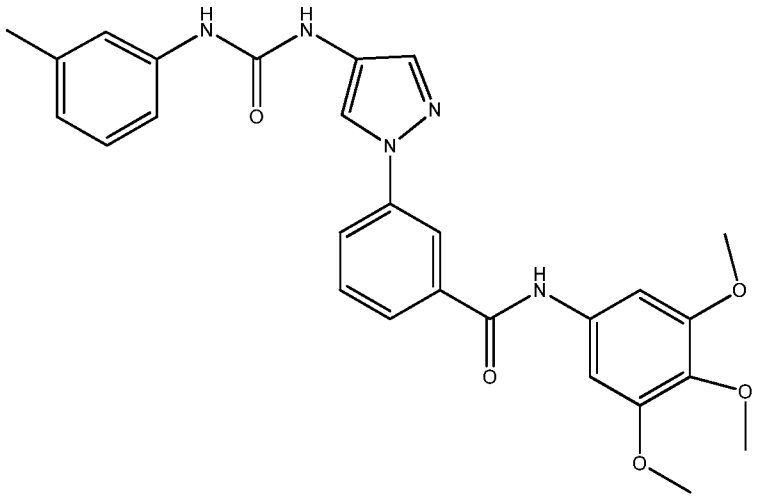
Formula (V)



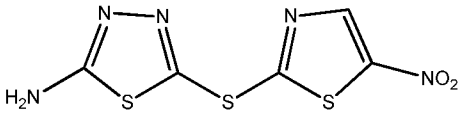
Formula (VI)



Formula (VII)



Formula (VIII)

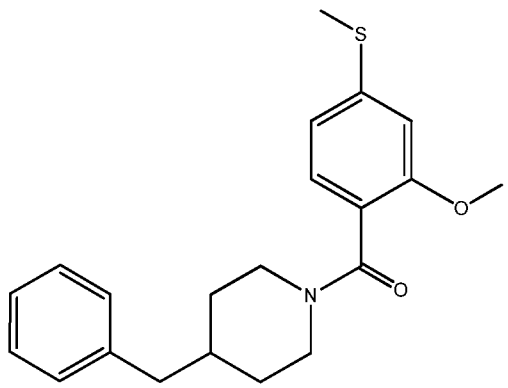


[0055] In some embodiments, the subject or cells to be treated are treated with a MAPK inhibitor as described herein. MAP kinases are serine/threonine-specific protein kinases that respond to extracellular stimuli (mitogens, osmotic stress, heat shock and proinflammatory cytokines) and regulate various cellular activities, such as gene expression, mitosis, differentiation, proliferation, and cell survival/apoptosis. MAP kinases can be classified as follows: extracellular signal-regulated kinases (ERK1, ERK2). The ERK1/2 (also known as classical MAP kinases) signaling pathway is preferentially activated in response to growth factors and phorbol ester (a tumor promoter), and regulates cell proliferation and cell differentiation.; c-Jun N-terminal kinases (JNKs), (MAPK8, MAPK9, MAPK10) also known as stress-activated protein kinases (SAPKs); p38 isoforms. p38-α (MAPK14), -β (MAPK11), -γ (MAPK12 or ERK6) and -δ (MAPK13 or SAPK4)), which are responsive to stress stimuli,, and are involved in cell differentiation and apoptosis; ERK5 (MAPK7), which has been found recently, is activated both by growth factors and by stress stimuli, and it participates in cell proliferation; ERK3/4. ERK3 (MAPK6) and ERK4 (MAPK4), which are structurally-related to atypical MAPKs possessing SEG motifs in the activation loop and displaying major differences only in the C-terminal extension; and ERK7/8.

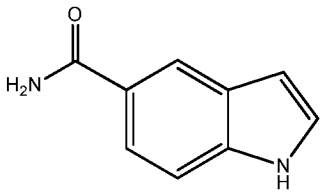
MAPK Inhibitors

[0056] Examples of MAPK inhibitors suitable for use in the methods described herein include, but are not limited to, any of the following compounds, or pharmaceutically acceptable salts or solvates thereof:

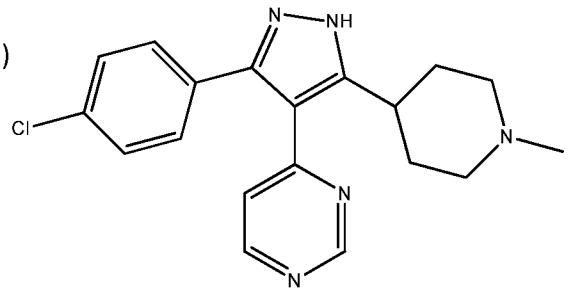
Formula (IX)



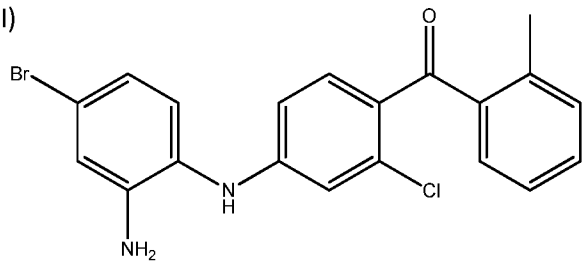
Formula (X)



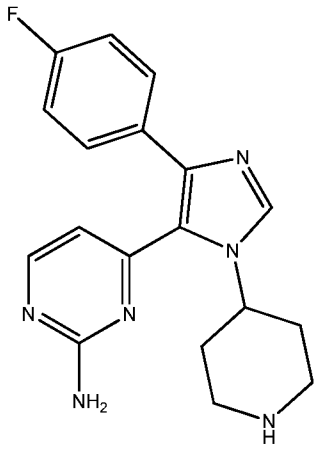
Formula (XI)



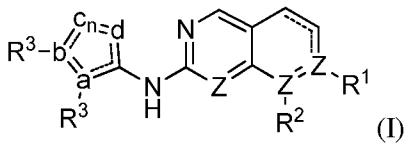
Formula (XII)



Formula (XIII)



[0057] In some embodiments, the subject or cells to be treated for a TDP-43 proteinopathy are treated with a compound having the structure of Formula (I):



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R^1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R^2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

each R^3 is not present or is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, and SO_2R^x;

each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

each Z is selected from C and N;

a is selected from C, N, O, and S;

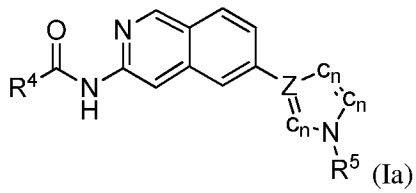
b is selected from C, N, O, and S;

c is selected from C, N, O, and S;

n is zero, one, or 2; and

d is selected from C, N, O, and S, when n is one or 2; or d is selected from O and S, when n is zero.

[0058] In some embodiments, the subject or cells to be treated for a TDP-43 proteinopathy are treated with a compound having the structure of Formula (Ia):



(Ia)

wherein:

R^4 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, and OR^x;

R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

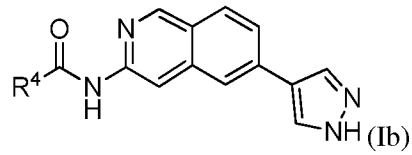
Z is selected from C and N;

c is selected from C, N, O, and S;

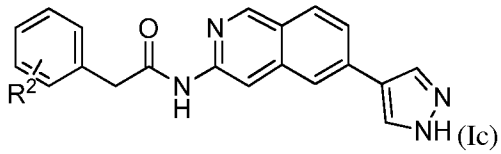
n is zero, one, or 2; and

R^5 is not present or is selected from hydrogen, alkyl, aryl, and heterocyclic.

[0059] In some embodiments, the subject or cells to be treated for a TDP-43 proteinopathy are treated with a compound having the structure of Formula (Ib) or Formula (Ic):



(Ib)



(Ic)

wherein:

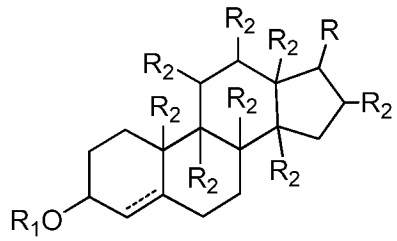
R^4 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, and OR^x;

R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

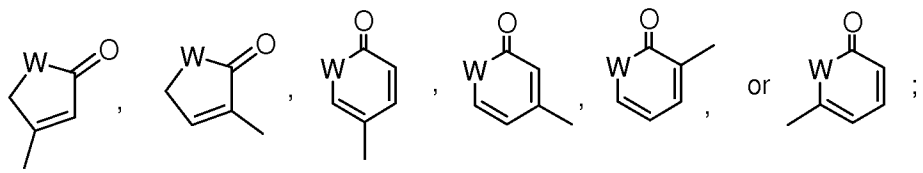
R^2 is selected from alkyl, substituted alkyl, alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino. aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-aryl, and $-SO_2$-heteroaryl.

[0060] In some embodiments, the subject or cells to be treated for a TDP-43 proteinopathy are treated with a cardiac glycoside compound having the structure of Formula (XIV):



(XIV)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R is  ;

R_1 is selected from a sugar species and hydrogen;

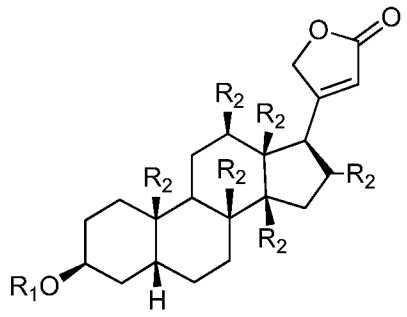
each R_2 is independently selected from OH, alkyl, hydrogen, alkyl, OH, and CHO; and

W is selected from O and NR_3; and

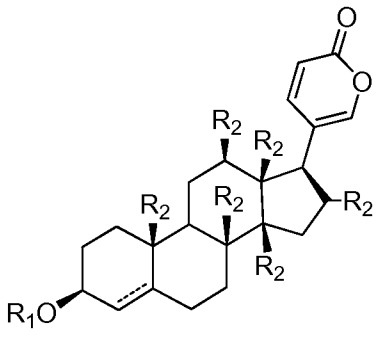
R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

[0061] Sugar species include, but are not limited to, monosaccharides, disaccharides, oligosaccharides, and polysaccharides. Suitable monosaccharides include the D-, L-, and DL-isomers of rhamnose, xylose, erythrose, erythrulose, threose, arabinose, lyxose, ribulose, ribose, xylose, xylulose, allose, altrose, fructose, galactose, glucose, gulose, fucose, idose, mannose, psicose, sorbose, tagatose, talose, N-acetylglucosamine, N-acetyl-galactosamine, galacturonic acid, and the like. The sugar species can be derivatized. The derivatized sugar species can be a deoxy-derivatized sugar species, a methylated sugar species, an acetylated sugar species, an amine-derivatized sugar species, and a phosphate-derivatized sugar species. Disaccharides comprise two monosaccharide units. Oligosaccharides and polysaccharides can comprise two, three, four, five, six, or more monosaccharide units.

[0062] In some cases, the structure of Formula (XIV) has the structure of Formula (XIVa) or (XIVb):



(XIVa)



(XIVb)

wherein:

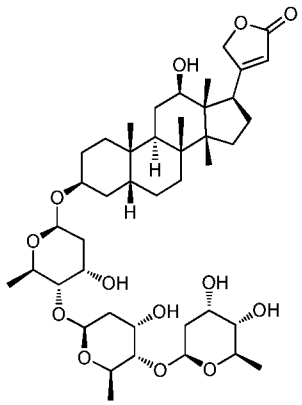
R_1 is selected from a sugar species and hydrogen;

each R_2 is independently selected from OH, alkyl, hydrogen, alkyl, OH, and CHO; and

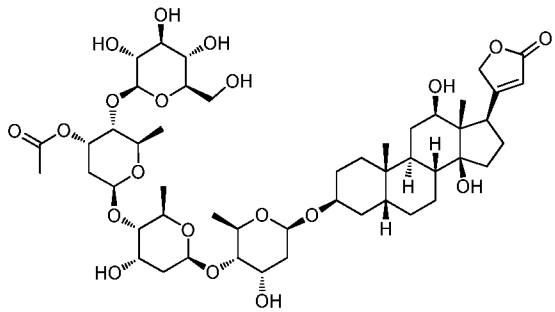
W is selected from O and NR_3; and

R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

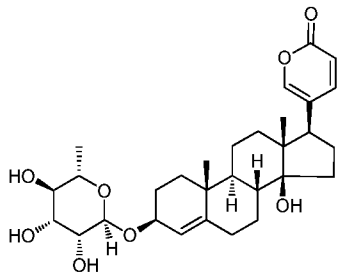
[0063] In other cases, the structure of Formula (XIV) has the structure of any of Formulas (XIVc) to (XIVg):



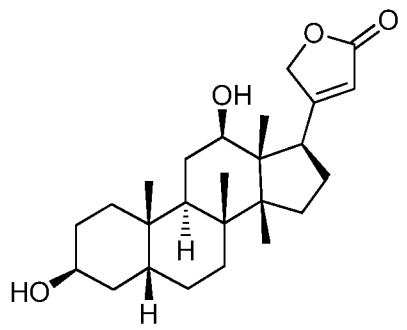
(XIVc)



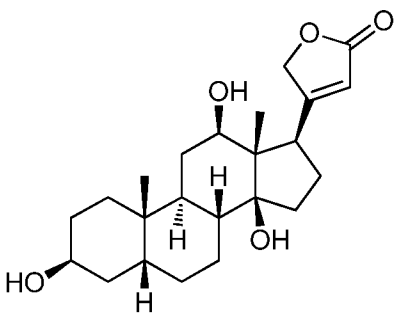
(XIVd)



(XIVe)

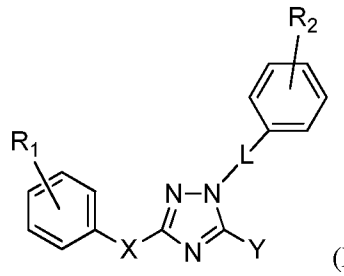


(XIVf)



(XIVg)

[0064] In some embodiments, the subject or cells to be treated for a TDP-43 proteinopathy are treated with a compound having the structure of Formula (XV):



(XV)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R_1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R_2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

L is not present or is selected from $-C(O)NR_4-$, $-C(O)NR_4$-alkyl-, $-C(O)-$, $-NR_4-$;

each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

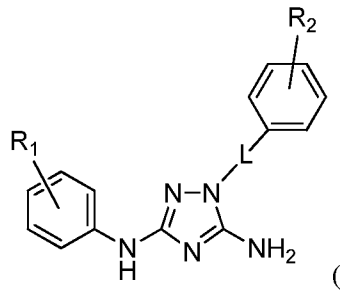
R_4 is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

X is selected from O and NR_3; and

R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

Y is selected from OH and NH_2.

[0065] In some cases, the compound having the structure of Formula XV has the structure of Formula (XVa):



(XVa)

wherein:

R_1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R_2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

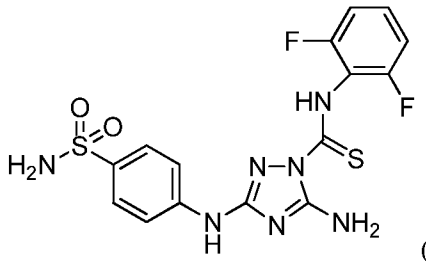
each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

L is not present or is selected from $-C(O)NR_4-$, $-C(O)NR_4$-alkyl-, $-C(O)-$, $-NR_4-$; and

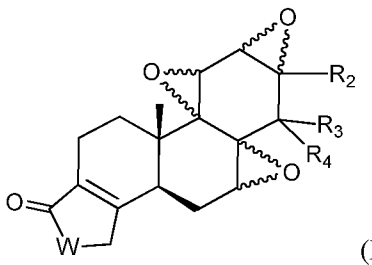
R_4 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

[0066] In some cases, the compound having the structure of Formula XV has the structure of Formula (XVb):



(XVb)

[0067] In other embodiments, the subject or cells to be treated for a TDP-43 proteinopathy are treated with a compound having the structure of Formula (XVI):



(XVI)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R^2 is selected from hydrogen, alkyl, alkylhydroxy, hydroxyalkyl, aryl, and heterocyclic;

R^3 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic;

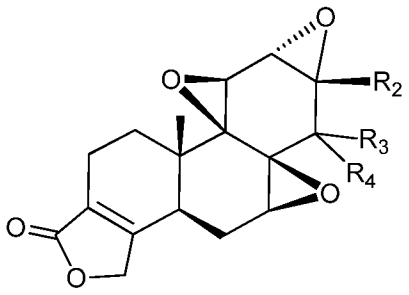
R^4 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic; or

R^3R^4 taken together is =O or NR^1; and

W is selected from O and NR^1;

each R^1 is independently selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic.

[0068] In some cases, the structure of Formula (XVI) has the structure of Formula (XVIa):



(XVIa)

wherein:

R^2 is selected from hydrogen, alkyl, alkylhydroxy, hydroxyalkyl, aryl, and heterocyclic;

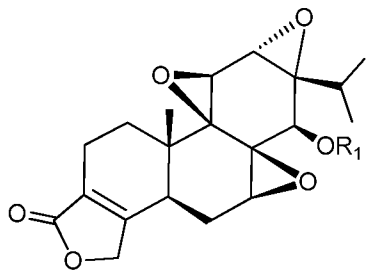
R^3 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic;

R^4 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic; or

R^3R^4 taken together is =O or NR^1; and

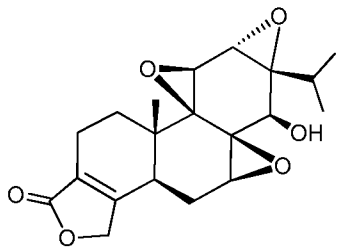
R^1 is selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic.

[0069] In other cases, the compound having the structure of Formula (XVI) has the structure of Formula (XVIb) or Formula (XVIc):



(XVIb)

wherein R^1 is selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic;



(XVIc)

[0070] In some embodiments, the subject or cells to be treated for a TDP-43 proteinopathy are treated with a composition (e.g., a pharmaceutical composition) comprising a therapeutically effective amount of a proteasome inhibitor (e.g., Bortezomib, MG132, Epoxomicin, or Lactacystin), a Topoisomerase I inhibitor (e.g., Topotecan and Camptothecin), a Topoisomerase II inhibitor (e.g., Doxorubicin and Epirubicin), a heat shock protein 90 (HSP-90) inhibitor (e.g., a) Geldanamycin, 17-AAG (17-N-

Allylamino-17-demethoxygeldanamycin), CAY10607), a 5-HT antagonist (e.g., Asenapine), a CDK inhibitor (e.g., Flavopiridol), or a transcription inhibitor (e.g., Actinomycin D).

Methods for Identifying Agents that Modulate TDP-43 Aggregation

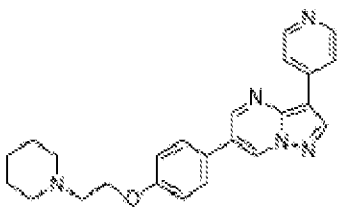
[0071] TDP-43 is expressed ubiquitously in cells, and in healthy neural progenitors and neurons is generally localized to the nucleus with a relatively even distribution. In contrast, in TDP-43 proteinopathies, e.g., ALS and FTD, TDP-43 is often mislocalized to the cytoplasm, and/or is found, in part, as intranuclear or cytoplasmic aggregates in motor neurons, or cortical neurons (e.g., layer V pyramidal cells), and other CNS cell types.

[0072] As described herein, a fraction of hiPSC lines generated from patients suffering from a TDP-43 proteinopathy, e.g., sALS, but not those from healthy subjects, consistently exhibit TDP-43 aggregates, e.g., intranuclear TDP-43 aggregates. Thus, hiPSC lines from TDP-43 proteinopathy patients (e.g., sALS patients) can be used to generate, on a large scale, cultures of neural progenitors and neurons (e.g., motor progenitors and motor neurons) that manifest TDP-43 aggregates. Such cultures are then used to identify agents that modulate the number of TDP-43 aggregates or the localization of TDP-43 within the cell. In some cases, an agent that modulates TDP-43 aggregation, decreases the fraction of cells in a population that exhibit TDP-43 aggregates, decreases the size of TDP-43 aggregates, or both. In other cases, an agent that modulates TDP-43 aggregation, may increase the fraction of cells in a population that exhibit TDP-43 aggregates, increases the size of TDP-43 aggregates, or both. In some embodiments, an agent that modulates TDP-43 aggregation, may selectively modify TDP-43 aggregation in one or more specific cell types, e.g., neurons, neural progenitors, motor progenitors, motor neurons, cortical neurons, or astrocytes. In other embodiments, an agent that modulates TDP-43 aggregation may modulate TDP-43 aggregation non-selectively.

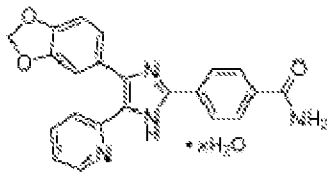
[0073] In some embodiments, where an hiPSC line has been shown to differentiate into TDP-43 aggregate-bearing neural progenitors or neurons, as described herein, the hiPSC line is expanded, differentiated, and plated at large scale (e.g., 384 well format) into neuronal cultures, which may contain both neural progenitors and neurons. In some embodiments, mixed neuronal cultures may be subjected to selection and sorting methods known in the art (e.g., FACS) to enrich for particular cell types, e.g., neurons, motor neurons, cortical neurons, neural progenitors, etc. Methods for differentiating, pluripotent stem cells into neural lineages are known in the art. See, e.g., Perrier, *et al* (2004), *PNAS-USA*, 101(34)12543-12548; Eiraku *et al* (2008), *Cell Stem Cell*, 3(5):519-532, and Chambers *et al* (2009), *Nat. Biotechnol.*, 27(3):275-280/ WO2010096496).

[0074] In one embodiment, an hiPSC line is differentiated into TDP-43 aggregate-bearing motor neuronal cultures, which contain both motor progenitors and motor neurons ("motor neuron cultures"). In some embodiments, the method used for differentiation of the hiPSC line into motor neuron cultures for screening is a modification of the "dual SMAD inhibitor" method described by Chambers *et al* (2009) *supra*, which has the advantage of converting hiPSCs to neural progenitors and motor neurons with great efficiency and consistency.

[0075] In an exemplary embodiment, hiPSC neural differentiation is initiated by replacing pluripotent stem cell growth medium (e.g., mTeSR™ medium) in a confluent 10 cm plate hiPSC culture plated on Matrigel™ (BD Biosciences) with hES medium containing 1μM Dorsomorphin and 10 μM 10 μM SB431542 (see structures below), and culturing in this medium for 3 days. hES Medium as described herein consists of Knock Out (KO)-DMEM, 10% KO-serum replacement, 10% Plasmanate, 1% Glutamax™, 1% Non-Essential Amino Acids (NEAA),
0.2% β-Mercaptoethanol.



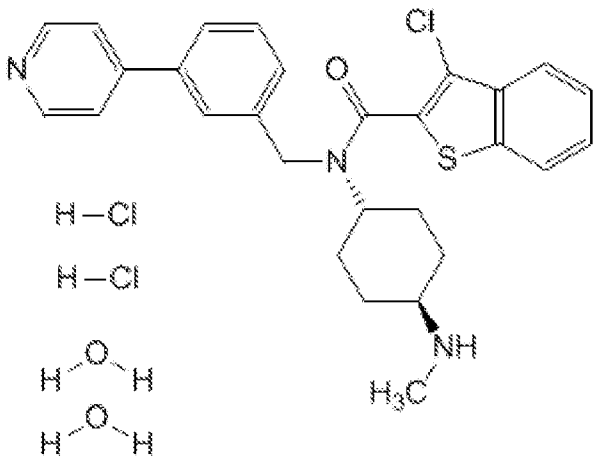
Dorsomorphin



SB431542

[0076] On Day 4, the medium is replaced with a mixed medium containing the above-mentioned hES Medium and N2 Base Medium at a ratio of 70:30, respectively, and containing final concentrations of Dorsomorphin (1 μM) and SB431542 (10 μM). "N2 Base Medium" consists of DMEM/F12 + Glutamax™, 1% (1X) N2 Supplement (Invitrogen, Carlsbad, CA), 0.16% (additional) D-Glucose, and 0.2 mM Ascorbic Acid. On Days 5 and 6, the ratio of hES:N2 Base Media is adjusted to 50:50, with final concentrations of Dorsomorphin, and SB431542 at 1 μM and 10 μM, respectively. On Days 7 and 8, the medium is switched to a mix of hES and Maturation Medium at a ratio of 50:50 with final concentrations of Dorsomorphin, and SB431542 at 1 μM and 10 μM, respectively, 1.5 μM All-Trans Retinoic Acid, and 200 nM Smoothened Agonist (SAG-see structure below) . "Maturation Medium" consists of DMEM/F12 + Glutamax™, 2% N2 Supplement (Invitrogen, Carlsbad, CA), 2% B27

Supplement (Invitrogen), 0.32% (additional) D-Glucose, 0.4 mM Ascorbic Acid, 2ng/ml brain derived neurotrophic factor (BDNF), 2 ng/ml, ciliary neurotrophic factor (CNTF), and 2 ng/ml glial cell derived neurotrophic factor (GDNF). On Days 9 and 10 the medium is switched to 100% Maturation Medium with final concentrations of Dorsomorphin, and SB431542 at 1 μM and 10 μM, respectively, 1.5 μM All-Trans Retinoic Acid, and 200 nM SAG.



SAG

[0077] During days 11-18, the medium is switch to Maturation Medium containing 1.5 μM All-Trans Retinoic Acid, and 200 nM SAG. After Day 18, the culture is split into a multiwell format. Typically one 10 cm dish will yield 5 to 10 384 well plates (at a plating density of approximately 7,500 cells/well). In one exemplary embodiment, cells are passaged by rinsing in calcium/magnesium-free PBS, followed by dissociation in enzyme-free dissociation buffer (Sigma, St. Louis, MO), trituration into a single cell suspension, pelleting, and resuspension in Maturation Medium containing 1.5 μM All-Trans Retinoic Acid, 200 nM SAG, and 10 μg/ml laminin. Cells may be plated, on poly-L-lysine/laminin coated plastic. In some embodiments, cells are imaged at least about 3 days to 10 days after plating, e.g., about 4, 5, 6, 7, 8, 9, or another period from at about 3 days to 10 days after plating.

[0078] For identification of agents that modulate TDP-43 aggregation, each well in a multiwall plate culture is incubated for about 8 hours to about 72 hours in the presence of a separate test agent. In some embodiments, each test agent is tested at a concentration of about 2 μM to about 30 μM in maturation medium, e.g., about 3, 5, 7, 10, 11, 14, 22, 25 μM, or another concentration from about 2 μM to about 30 μM in maturation medium. In some cases, medium with the test agent is replaced with fresh test agent after about 24 hours in cultures. Methods for automation and robotic handling of cell cultures for drug discovery are known in the art, and facilitate the use of methods described herein for screening of large

sets of test agents. Test agents, include, but are not limited to, small molecules, nucleic acids (e.g., RNAi, shRNAs, microRNAs, and cDNAs), antibodies.

[0079] A number of methods are known in the art for determining the distribution of proteins and their aggregation in specific cell types. Such methods include, but are not limited to, immunofluorescence microscopy (e.g., confocal microscopy, two-photon laser scanning microscopy, and epifluorescence microscopy), flow cytometry, immunohistochemistry, electron microscopy, and biochemical fractionation.

[0080] In some embodiments, a polyclonal or monoclonal antibody against full-length TDP-43, a TDP-43 fragment, or a TDP-43 phosphopeptide is used to visualize TDP-43 distribution and TDP-43 aggregates in motor progenitors and motor neurons in the above-described cultures by immunofluorescence confocal microscopy. In some embodiments, TDP-43 is co-immunostained with other markers to identify cell type, and subcellular localization. In one embodiment, TDP-43 is co-stained with Islet 1 (a transcription factor localized to the nucleus) to identify motor progenitors or immature motor neurons containing TDP-43 aggregates. In some embodiments, TDP-43 is co-stained with an antibody against HB9, a transcription factor typically expressed in mature motor neurons. In other embodiments, both Islet1 and HB9 are detected along with TDP-43. Typically, the nucleus is co-stained with a DNA-binding dye such as 4',6-diamidino-2-phenylindole (DAPI) or 2'-[4-ethoxyphenyl]-5-[4-methyl-1-piperazinyl]-2,5'-bi-1H-benzimidazole trihydrochloride trihydrate (Hoechst 33342).

[0081] In one exemplary embodiment, TDP-43 staining for aggregates in the motor neuron cultures described herein is performed as follows. Cultures are fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) at room temperature for 20 minutes, after which the fixation solution is removed, and the fixed cultures are washed twice with PBS. After washing, the fixed cells are then incubated in blocking solution consisting of PBS with 0.1% Triton X-100 and 10% Donkey Serum with rocking for one hour at room temperature. Subsequently, the cultures are incubated in blocking solution containing primary antibodies, and incubated at 4 °C overnight. After the primary antibody incubation period, the fixed cultures are washed three times in PBS containing 0.01% Triton X-100. The cultures are then incubated in secondary antibodies (dilution 1:300) in PBS containing 0.01% Triton X-100 and 5% Donkey Serum at room temperature in the dark with rotation. Subsequently, the fixed cultures are washed three times (room temperature) in PBS containing 0.01% Triton X-100, and then incubated in Hoechst diluted 1:1000 in PBS containing 0.01% Triton X-100. Finally, the fixed cultures are washed three times in PBX at room temperature prior to visualization. In some embodiments, other primary antibodies include a monoclonal antibody against human Islet 1 (dilution of 1:1000), and/or a monoclonal antibody against human HB9 (dilution of 1:100). In some embodiments, the entire antibody staining process is automated,

and multichannel image acquisition is performed on a high throughput confocal microscopy imager, e.g., the ImageXpress Ultra platform (Molecular Devices, Sunnyvale, CA).

[0082] In one embodiment, imaging data from TDP-43 immunofluorescence staining in combination with other immunofluorescence staining of markers of motor progenitor and/or motor neurons (e.g., Islet 1 and HB9) is processed through imaging algorithms to automatically locate and quantify nuclear markers in a dense, heterogeneous populations of cells, and to determine the fractions of cells having at least one aggregate, separately for motor neurons and other cell types. In some embodiments, individual cells are located and quantified using algorithms based on grayscale morphology as described, e.g., in (Dougherty *et al* (2003), "Hands on Morphological ImageProcessing," SPIE Press, The International Society for Optical Engineering, Bellingham, WA ; and Volfson *et al* (2008), *PNAS-USA*, 105(40):15346-15351). In one embodiment, where TDP-43 aggregates are known to be intranuclear for a given hiPSC line-derived neural cell, information collected from all three channels is combined and used to identify nuclei, including cases when they partially overlap. Once nuclei have been identified, the average intensity is determined in the TDP-43 and ISLET/HB9 marker channels. Anti-TDP-43 immunofluorescence also permits the location of TDP-43 aggregates and quantification of their average intensity, local contrast, and size. Subsequently, identified aggregates which fall out of recognized nuclei are filtered out, whereas a nucleus is classified as aggregate-positive when at least one aggregate is located within the nuclear area. Subsequently, nuclei are classified as ISLET/HB9 positive or negative based on their average nuclear intensity. Finally, information obtained from the previous steps is combined to quantify various subpopulations of cells, that is ISLET/HB9 positive/negative, aggregate positive/negative as well as double positive. Quantification may include testing possible differences in average intensities in all three channels, differences in apparent areas of the nuclei, differences in fraction of aggregate positive (negative) and ISLET/HB9 positive (negative) within all identified nuclei, and fraction of double positive within each subcategory (fraction of ISLET/HB9 positive/negative within aggregate positive/negative and vice-versa). Algorithms may be implemented in Matlab (Mathworks Inc., Natick, MA). Thus, based on the above-described analysis, a test-reagent may be determined to reduce one or more of the number, intensity, or size of TDP-43 aggregates in one or more cell types, increase them, or have no effect, as compared to these properties in cells of the same type (e.g., motor progenitors, and motor neurons differentiated from the same patient hiPSC line) in the absence of the test agent.

IV. Compositions

Overview

[0083] The compositions described herein are drawn to hiPSC lines generated from patients suffering from a TDP-43 proteinopathy (e.g., sporadic ALS or FTD), characterized by the unusual property that a fraction of motor neurons or neural progenitors obtained by differentiating the human induced pluripotent stem cell lines comprise one or more TDP-43 aggregates (e.g., intranuclear TDP-43 aggregates). As described herein, unexpectedly, such hiPSC lines are found with a frequency of about 18%. Thus, without undue effort, patient hiPSC lines that yield TDP-43 aggregate-positive neural progenitors and neurons may be identified from a panel of TDP-43 proteinopathy patient (e.g., sALS) hiPSC lines by the methods described herein. Other compositions described herein also include populations of cells containing viable neural progenitors (e.g., motor progenitors), neurons (e.g., motor neurons), or both, generated from where a fraction of such cells exhibit a TDP-43 aggregate, and such cells were obtained by differentiation of an hiPSC line generated from a patient suffering from a TDP-43 proteinopathy. In some embodiments, differentiation of hiPSCs is performed as described above under "Methods for Identifying Agents that Modulate TDP-43 Aggregation." In some embodiments, the fraction of TDP-43 aggregate-positive cells obtained by neural differentiation of such lines is at least about 10% to about 50%, e.g., 12%, 15%, 18%, 20%, 23%, 30%, 35%, 42%, or another fraction from about 10% to about 50% of the differentiated cells. In some cases, the fraction of TDP-43-aggregate-positive /Islet-1-positive cells in the total differentiated cell population is greater than the fraction of TDP-43 aggregate-positive/Islet-1-negative cells in the differentiated cell population.

[0084] Methods for generating hiPSC lines by introduction of exogenous "reprogramming factors" using integrating and non-integrating methods are known in the art. See, e.g., Dimos *et al* (2008), *Science*, 321(5893):1218-1221, Chan *et al* (2009), *Nature Biotechnol*, 27(11):1033-1037; US Patent Application No. 20090191159; and Nishimura *et al* (2011), 286(6):4760-4771. In some embodiments, fibroblasts from a patient diagnosed as suffering from a TDP-43 proteinopathy (e.g., sALS or FTD) are obtained by culturing a patient skin biopsy.

[0085] Examples of suitable reprogramming factor genes for generating hiPSC lines from fibroblasts include, but are not limited to genes encoding a polypeptide that comprises an amino acid sequence at least 80% identical, e.g., at least 85%, 88%, 90%, 95%, 97%, or another percent identical to the amino sequence of any of the following human or mouse sequences: Oct 4 (GenBank Accession Nos. NP_002692 and NP_038661.2, respectively), Sox2 (GenBank Accession Nos. NP_003097.1 and AAH57574, respectively), Klf4 (GenBank Accession Nos. NP_004226.3 and NP_034767.2, respectively), c-Myc (NP_002458.2 and NP_034979, respectively), Nanog (AY230262.1 and NP_082292.1, respectively), and

Lin-28 (NP_078950.1 and NP_665832.1, respectively). In some embodiments, the encoded reprogramming factors may also include human or mouse activation-induced cytidine deaminase (AID), (GenBank Accession Nos. (NP_065712.1 and NP_033775.1, respectively). In some embodiments, the encoded reprogramming factor amino acid sequences are from human. In other embodiments, the encoded sequences are from mouse. In some embodiments, a nucleic acid expression vector encodes the human ortholog of any of Oct 4, Sox2, Klf4, c-Myc, Nanog, or Lin-28. In other embodiments, a nucleic acid expression vector encodes the mouse ortholog of any of Oct 4, Sox2, Klf4, c-Myc, Nanog, or Lin-28.In some embodiments, an expression cassette is a polycistronic expression cassette that encodes the amino acids sequences of multiple reprogramming factors, the expression of which is under the control of the same promoter. Such polycistronic expression cassettes may include at least two, three, four, five, or six reprogramming factors. In some cases, an expression cassette includes the open reading frames for Oct 4 and Sox2. In other cases, the expression cassette includes the open reading frames for Oct 4, Sox2, and Klf4. In other embodiments, the expression cassette includes the open reading frames for Oct 4, Sox2, Klf4, and c-Myc. In further embodiments, the expression cassette includes the open reading frames for Oct 4, Sox2, Nanog, and Lin-28. In some embodiments, a polycistronic expression cassette for expression of multiple reprogramming factors contains the sequence encoding the 2A peptide between the sequences encoding the various reprogramming factors. A polycistronic expression cassette may contain the sequences of reprogramming factors from 5' to 3' in any order. In one embodiment DNA expression vectors comprise a single DNA expression vector encoding reprogramming factors consisting of Oct4, Sox2, Klf4, and c-Myc, where the order of the sequences if from 5' to 3' c-Myc, Klf4, Oct4, Sox2. In some cases, a polycistronic expression cassette comprises a nucleic acid sequence encoding reprogramming factors in the order from 5' to 3' c-Myc, Klf4, Oct4, Sox2 with intervening 2A sequences.

[0086] Various combinations of exogenous reprogramming factors can be used to reprogram human fibroblasts. In some embodiments, the exogenous reprogramming factors to be expressed include the four factors Oct4, Sox2, Klf4, and c-Myc. In some embodiments the exogenous reprogramming factors include Oct4, Sox2, Klf4, c-Myc, and Nanog. In other embodiments, the exogenous reprogramming factors include (i) the four reprogramming factors Oct4, Sox2, Klf4, c-Myc, but without additional exogenous reprogramming factors, or (ii) the five reprogramming factors Oct4, Sox2, Klf4, c-Myc, and Nanog, but without additional exogenous reprogramming factors. In other embodiments, the four exogenous reprogramming factors include Oct4, Sox2, Nanog, and Lin-28, or Oct4, Sox2, Nanog, and Lin-28, but without additional exogenous reprogramming factors.

[0087] In further embodiments, the exogenous reprogramming factors include the three reprogramming factors Oct4, Sox2, and Klf4; or include Oct4, Sox2, and Klf4, but without additional exogenous reprogramming factors.

[0088] Suitable media for hiPS culture, particularly under feeder cell-free conditions, for the methods described herein include, but are not limited to, mTeSR™ (available, e.g., from StemCell Technologies, Vancouver, Canada), See, e.g., Ludwig *et al*, (2006), *Nat Biotechnol*., 24(2):185-187. In other cases, alternative culture conditions for growth of hiPS cells are used, as described for human ES cells in, e.g., Skottman et al., (2006), *Reproduction*, 132(5):691-698. Typically, culture medium suitable for maintenance and passaging of hiPS cells includes fibroblast growth factor (FGF-2) at a concentration of about 5 ng/ml to about 100 ng/ml. In some cases, hiPS cells may be cultured under xeno-free conditions, e.g., in "RegES" medium as described in Rajala *et al* (2010), *PLoS One*, 5(4):e10246. In some embodiments, the transfected iPSCs are plated on mouse embryonic fibroblast (MEF) feeder cells in hES culture medium.

[0089] In some cases after about 20 days to about 40 days of maintaining transfected iPSCs in hiPS cell medium (e.g., mTeSR™), e.g., about 21 days, 22 days, 24 days, 26 days, 30 days, 32 days, 34 days, 36 days, or another period from about 20 days to about 40 days, cultures are monitored for the presence of adherent colonies of hiPSCs, which typically are made up of small cells having a high nucleus to cytoplasm ratio. Individual colonies are then picked and transferred individually to new wells for subcloning and characterization.

Analysis of hiPS Cells

[0090] Methods for identifying hiPS cells and hiPS cell colonies are known in the art. For example, putative iPS cell colonies may be tested for alkaline phosphatase (ALP) activity, and if positive, may then be assayed for expression of a series of human embryonic stem cell marker (ESCM) genes including, but not limited to, Nanog, E-Cadherin, DNMT3b, TDGF1, Lin-28, Dnmt3b, Zfp42, FoxD3, GDF3, CYP26A1, TERT, Oct 3/4, Sox2, Rex1, Sall4, and HPRT. See, e.g., Assou *et al*., (2007), *Stem Cells*, 25:961-973. Many methods for gene expression analysis are known in the art. See, e.g., Lorkowski *et al*., (2003), *Analysing Gene Expression, A Handbook of Methods: Possibilities and Pitfalls*, Wiley-VCH. Examples of suitable nucleic acid-based gene expression assays include, but are not limited to, quantitative RT-PCR (qRT-PCR), microarray hybridization, dot blotting, RNA blotting, RNAse protection, and SAGE.

[0091] In some embodiments, levels of ESCM gene mRNA expression levels in putative iPS cells colonies are determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Putative iPS cell colonies are harvested, and total RNA is extracted using the "Recoverall total nucleic acid isolation kit for formaldehyde- or paraformaldehyde-fixed, paraffin-embedded (FFPE) tissues" (manufactured by

Ambion, Austin, TX). In some instances, the colonies used for RNA extraction are fixed colonies, e.g., colonies that have been tested for alkaline phosphatase (ALP) activity. The colonies can be used directly for RNA extraction, i.e., without prior fixation. In an exemplary embodiment, after synthesizing cDNA from the extracted RNA, the target gene is amplified using the TaqMan® PreAmp mastermix (manufactured by Applied Biosystems, Foster City, CA). Real-time quantitative PCR is performed using an ABI Prism 7900HT using the following PCR primer sets (from Applied Biosystems) for detecting mRNA of the above-mentioned ESCM genes: Nanog, Hs02387400_g1, Dnmt3b, Hs00171876_ml, FoxD3, Hs00255287_s1, Zfp42, Hs01938187_s1, TDGF1, Hs02339499_g1, TERT, Hs00162669_m1, GDF3, Hs00220998_m1, CYP26A1, Hs00175627_m1, GAPDH, Hs99999905_m1). Putative hiPS cell colonies may be assayed by an immunocytochemistry method for expression of protein markers including, but not limited to, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, CD9, CD24, Thy-1, and Nanog. A wide range of immunocytochemistry assays, e.g., fluorescence immunocytochemistry assays, are known as described in, e.g., Harlow *et al.*, (1988), *Antibodies: A Laboratory Manual* 353-355, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, and see also, *The Handbook -A Guide to Fluorescent Probes and Labeling Technologies* (2004), Molecular Probes, Inc., Eugene, OR. In some cases, immunofluorescence staining is followed by quantitation of the number of cells immunopositive for one or more of the above-mentioned ES-cell protein markers. Such quantitative methods include, but are not limited to flow cytometry and image cytometry.

[0092] It is generally believed that pluripotent stem cells have the ability to form a teratoma, comprising ectodermal, mesodermal, and endodermal tissues, when injected into an immunocompromised animal. Induced cells or induced pluripotent stem cells (iPS) or ES cell-like pluripotent stem cells may refer to cells having an in vitro long-term self-renewal ability and the pluripotency of differentiating into three germ layers, and said pluripotent stem cells may form a teratoma when transplanted into a test animal such as mouse.

[0093] The induced cells may be assessed for pluripotency in a teratoma formation assay in an immunocompromised animal model. The immunocompromised animal may be a rodent that is administered an immunosuppressive agent, e.g., cyclosporin or FK-506. For example, the immunocompromised animal model may be a SCID mouse. About 0.5 x 10^6 cells to about 2.0 x 10^6 cells e.g., 0.6 x 10^6 cells, 0.8 x 10^6 cells, 1.0 x 10^6 cells, 1.2.times x 10^6 cells, 1.5.times x 10^6 cells, 1.7 x 10^6 cells, or other number of induced cells from about 0.5 x 10^6 cells to about 2.0 x 10^6 cells induced cells/mouse may be injected into the medulla of a testis of a 7- to 8-week-old immunocompromised animal. After about 6 to about 8 weeks, the teratomas are excised after perfusing the animal with PBS followed by 10% buffered formalin. The excised teratomas are then subjected to immunohistological

analysis. One method of distinguishing human teratoma tissue from host (e.g., rodent) tissue includes immunostaining for the human-specific nuclear marker HuNu. Immunohistological analysis includes determining the presence of ectodermal (e.g., neuroectodermal), mesodermal, and endodermal tissues. Protein markers for ectodermal tissue include, but are not limited to, nestin, GFAP, and integrin β1. Protein markers for mesodermal tissue include, but are not limited to, collagen II, Brachyury, and osteocalcin. Protein markers for endodermal tissue include, but are not limited to, alpha-fetoprotein (alpha-FP) and HNF3beta.

EXAMPLES

Example 1 Generation of hiPSC Lines from Sporadic ALS Patients and their Differentiation into Motor Neuron Progenitors and Motor Neurons

[0094] The single most dominant form of pathology across sporadic ALS (sALS) patients (identified post-mortem) has been the presence of TDP-43 pathology including hyperphosphorylated and ubiquitinated TDP-43 nuclear and cytoplasmic aggregates, loss of nuclear TDP-43, as well as cleavage of TDP-43 into smaller fragments in patient motor neurons and TDP-43 proteinopathy has been suggested to be causal for ALS. TDP-43 pathology has also been discovered in patients with other adult-onset neurodegenerative diseases including frontotemporal dementia (FTD), Alzheimers disease, and Lewy body with dementia suggesting that it may play a central role in many neurodegenerative diseases. The molecular mechanism underlying formation of TDP-43 pathology is unknown and mutations in TDP-43 are present in only 5% of sALS cases while nearly all sALS patients studied so far show TDP-43 pathology. It was surmised that reprogramming of patient cells and differentiation into motor neurons that show a disease phenotype could provide us with a disease model that can be used for target identification, drug discovery, and mechanistic studies. While cellular reprogramming has been achieved for familial ALS patients with SOD1 mutations, no phenotype has been reported in these cells (Dimos *et al* (2008), *Science*, 321(5893):1218-1221). In order to gain insight into sALS, sALS patient fibroblasts were reprogrammed into induced pluripotent stem cells (iPSC) and differentiated these cells into motor neurons to create a cellular model for this disease. Here is provided a description of the unexpected result that, notwithstanding the fact that sALS is generally a late onset neurodegenerative disease, a fraction of iPSC lines derived from sALS patients and differentiated into motor neurons show de novo TDP-43 pathology and therefore are a novel cellular model for studying ALS and TDP-43 proteinopathy.

[0095] Retroviruses carrying human KLF4, SOX2, OCT4, and cMYC (essentially as described in Dimos *et al, supra*) were used to generate iPSC clones from a cohort of individuals including healthy subjects, fALS, and sALS patients as listed in Table 1. iPSC clones were characterized for morphology consistent with human pluripotent cells; and clones were identified with a normal karyotype that express the following pluripotency markers to levels equivalent to human ES cell lines: CDH1, CYLIN D1, DNMT3B, DPPA4, FOXD3, GDF3, LEFTY1, LEFTY2, LIN28, NANOG, NODAL, SALL4, TDGF1, TDGF1&3, TERT,

UTF1, ZFP42, ZNF206 (Table 1, Figure 1A). iPSC lines were also assessed by immunofluorescence and flow cytometry for pluripotency markers (Figure 2).

TABLE 1

Patient information						iPSC-clone information					
rian Clone	*Health status*	*Mutation*	*Gender*	*Age*	*Age of disease onset*	*Karyotype*	*Viral gene silencing*	*Pluripotency Gene Expression*	*EB Differentiation*	*MN Differentiation*	*TDP-43 stain in MN*
JRN.0260.1						Normal	Klf4				Normal
	Healthy	NA	M	69	NA		High				
J.0014.7	Healthy	NA	F	ND	NA	Normal	ND	ND	ND		Normal
J.0165.1	Healthy	NA	F	86	NA	Normal					Normal
						Abnorma					Normal
J.0261.5	Healthy	NA	F	52	NA	l			ND		
J.0013.11	Healthy	NA	M	10	NA	Normal	ND	ND			Normal
J.0013.13	Healthy	NA	M	10	NA	Normal					Normal
J.0266.7	Healthy	NA	F	57	NA	ND			ND		Normal
J.0209.4	fALS	Unknown	M	51	ND	Normal			ND		Normal
J.0273.1	fALS	FUS	F	51	43	Normal					Normal
J.0273.2	fALS	FUS	F	51	43	Normal			ND		Normal
		SOD1									Normal
J.0049.1	fALS	Neg.	M	20s	20s	Normal			ND		
		SOD1									Normal
J.0049.4	fALS	Neg.	M	20s	20s	Normal			ND		
J.0298.1	fALS	Unknown	M	65	58	Normal			ND		Normal
						Abnorma					Normal
J.0298.9	fALS	Unknown	M	65	58	l			ND		
		TDP43				ND	Klf4				Normal
J.0270.4	fALS	A315T	M	53	48		High		ND		
		TDP43				ND	Klf4				Normal
J.0270.7	fALS	A315T	M	53	48		High		ND		
		TDP43				ND					Normal
J.0270.9	fALS	A315T	M	53	48		ND	ND	ND		
		SOD1									Normal
J.0028.30	fALS	N139K	F	48	46	Normal					
		SOD1									Normal
J.0028.13	fALS	N139K	F	48	46	Normal					
J.0268.3	fALS	FUS	M	39	ND	Abnorma			ND		Normal

						I				
		SOD1				ND				Normal
J.0032.2	fALS	A4V	F	58	57		ND	ND	ND	
		SOD1					Oct4			Normal
J.0032.4	fALS	A4V	F	58	57	Normal	High		Ecto+,Endo+	
		SOD1					Oct4			Normal
J.0032.5	fALS	A4V	F	58	57	Normal	High		Ecto+,Endo+	
		SOD1								Normal
J.0032.7	fALS	A4V	F	58	57	Normal				
		SOD1								Normal
J.0032.9	fALS	A4V	F	58	57	Normal				
J.0191.1	sALS	Unknown	F	53	41	Normal			ND	Normal
J.0191.2	sALS	Unknown	F	53	41	Normal			ND	Normal
							Myc			Normal
J.0191.3	sALS	Unknown	F	53	41	Normal	High		ND	
J.0192.1	sALS	Unknown	F	34	31	ND	ND	ND	ND	Normal
J.0192.2	sALS	Unknown	F	34	31	ND	ND	ND	ND	Normal
J.0192.3	sALS	Unknown	F	34	31	ND	ND	ND	ND	Normal
J.0194.1	sALS	Unknown	M	63	59	ND	ND	ND	ND	Normal
J.0194.4	sALS	Unknown	M	63	59	ND	ND	ND	ND	Normal
J.0194.5	sALS	Unknown	M	63	59	ND	ND	ND	ND	Normal
J.0198.1	sALS	Unknown	M	46	43	ND	ND	ND	ND	Normal
J.0198.4	sALS	Unknown	M	46	43	ND	ND	ND	ND	Normal
J.0171.2	sALS	Unknown	F	61	61	Normal	ND	ND	ND	Normal
						Abnorma				Normal
J.0171.3	sALS	Unknown	F	61	61	l	ND	ND	ND	
							Oct4			Normal
J.0171.5	sALS	Unknown	F	61	61	Normal	High		ND	
J.0171.7	sALS	Unknown	F	61	61	Normal			ND	Normal
J.0188.5	sALS	Unknown	F	68	66	ND			ND	Normal
J.0188.6	sALS	Unknown	F	68	66	Normal			ND	Normal
J.0188.7	sALS	Unknown	F	68	66	Normal			ND	Normal
							Myc			Normal
J.0047.3	sALS	Unknown	M	40s	40s	Normal	High		ND	
J.0048.1	sALS	Unknown	F	50s	50s	Normal				Abnormal
							Oct4			Abnormal
J.0048.2	sALS	Unknown	F	50s	50s	Normal	High		ND	
J.0048.3	sALS	Unknown	F	50s	50s	Normal			ND	Normal
J.0048.4	sALS	Unknown	F	50s	50s	Normal			ND	Normal

J.0048.5	sALS	Unknown	F	50s	50s	Normal			ND	Normal
J.0360.1	sALS	Unknown	F	62	58	ND	ND	ND	ND	Abnormal
J.0360.2	sALS	Unknown	F	62	58	ND	ND	ND	ND	Abnormal
J.0360.3	sALS	Unknown	F	62	58	ND	ND	ND	ND	Abnormal
J.0360.4	sALS	Unknown	F	62	58	ND	ND	ND	ND	Abnormal
J.0206.2	sALS	Unknown	M	70	54	ND	ND	ND	ND	Normal
J.0206.3	sALS	Unknown	M	70	54	ND	ND	ND	ND	Normal
J.0347.1	sALS	Unknown	F	55	53	ND	ND	ND	ND	Normal
J.0347.3	sALS	Unknown	F	55	53	ND	ND	ND	ND	Normal
J.0211.13	sALS	Unknown	F	67	61	ND	ND	ND	ND	Normal
J.0211.2	sALS	Unknown	F	67	61	ND	ND	ND	ND	Normal
J.0205.7	sALS	Unknown	M	56	53	ND			ND	Normal
						ND	Klf4			Normal
J.0205.8	sALS	Unknown	M	56	53		High		ND	
J.0199.2	sALS	Unknown	F	55	47	ND	ND	ND	ND	Normal
J.0199.6	sALS	Unknown	F	55	47	ND	ND	ND	ND	Normal
J.0199.7	sALS	Unknown	F	55	47	ND	ND	ND	ND	Normal
J.0204.7	sALS	Unknown	M	56	51	Normal			ND	Normal
J.0204.9	sALS	Unknown	M	56	51	Normal			ND	Normal
J.0015.2	sALS	Unknown	F	34	29	ND				Abnormal
J.0015.5	sALS	Unknown	F	34	29	ND	ND	ND	ND	Normal
J.0015.8	sALS	Unknown	F	34	29	ND	ND	ND	ND	Normal
J.0007.2	SMA	Type I	ND	ND	ND	Normal	ND	ND	ND	Normal
J.0008.3	SMA	Type I	ND	ND	ND	Normal			ND	Normal

y

)= not determined

. = not applicable

Verified

rmal = nuclear staining with Proteintech polyclonal Ab, no aggregates

.S = familial ALS

_S = sporadic ALS

lA= Spinal Muscular Atrophy

[0096] iPSC clones that passed quality control were differentiated into motor neurons using a dual SMAD inhibition method (described in WO2010096496) and further characterized for neuronal markers. iPSC were differentiated towards the neural lineage for 18 days after which they were passaged and analyzed for neuronal markers in at least two time points: one week and two weeks after passaging (approximately 25 and 32 days after neural induction). Motor neuron differentiation was assessed by immunohistochemistry for motor neuron markers ISLET and HB9. (Figure 1B and Figure 3). The iPSC-MN cultures were mixtures of progenitors and neurons and expressed motor neuron markers as early as 2 days after passage (day 20) the earliest time expression was assessed. iPSC-MN cultures one week after passage (day 25) contained many Islet and HB9-expressing cells (Figure 1C). In order to test the function of neurons, iPSC-MN cultures were assessed for neuronal activity using calcium imaging. The iPSC-MN did not show neuronal activity during the first 1-2 months post differentiation but showed calcium spikes after approximately 3 months in culture. Calcium transients were blocked by the voltage-gated sodium channel blocker TTX indicating calcium spikes are due to action potentials . Synchronous calcium spikes were also observed in neurons in close proximity to one another indicating the presence of a neuronal network and synapses in iPSC-MN cultures. These data indicated that human fibroblasts could be reprogrammed into pluripotent stem cells that differentiated into neurons and that these neurons had phenotypic and functional properties consistent with those of motor neurons.

[0097] TDP-43 and FUS are RNA binding proteins that have been shown to be important for regulation of RNA splicing indicative of a role for RNA metabolism in ALS. Nearly all sporadic ALS patients show some form of TDP-43 pathology including hyperphosphorylated nuclear and cytoplasmic TDP-43 positive inclusions, aggregates that are ubiquitin-positive, nuclear clearing of TDP-43, and cleavage of TDP-43 into smaller fragments. Previous studies indicated that TDP-43 pathology is absent from fALS patients with SOD1 mutations suggesting different mechanisms in familial and sporadic forms of ALS. Many studies have shown that TDP-43 aggregates are toxic to motor neurons, yet it is not known at what point during the course of ALS TDP-43 pathology appears in motor neurons or whether TDP-43 aggregates form before symptoms appear. Given that presence of aggregates in patient tissue is usually accompanied by a loss of normal nuclear staining, a loss of function for TDP-43 in the nucleus as well as a toxic gain of function in the cytoplasm has been proposed for the cause of TDP-43 toxicity. In order to identify TDP-43 pathology in patient iPSC-MN, iPSC-MN cultures differentiated from a panel of iPSC clones representing healthy individuals, familial ALS, sporadic ALS, and 2 spinal muscular atrophy (SMA) patients were stained; and differences in TDP-43 subcellular localization were examined. All

iPSC-MN cultures were stained with a rabbit polyclonal antibody (Proteintech Inc.) that has been used to detect TDP-43 pathology in human tissue in other studies. It was found that all cells in iPSC-MN cultures express TDP-43 with a predominantly nuclear pattern consistent with previous reports that TDP-43 is a nuclear protein (Figures 2 and 3). ISLET1-positive motor neurons had higher levels of TDP-43 as compared to other cells within these mixed cultures suggesting TDP-43 plays an important role in motor neurons (Figs. 2 and 3). Among the cultures that were screened for TDP-43 pathology (Table 1), three sporadic ALS patients (3 out of 16 sALS patients) were identified for which motor neuron cultures showed *de novo* TDP-43 aggregation (Fig. 3). In these three patients, in addition to the normal nuclear staining, TDP-43 antibody also stained bright juxta-nuclear round structures that are morphologically similar to nuclear inclusions observed in ALS and FTD patient postmortem spinal cord and brain in other studies (Figure 2, 3, and 4 arrows). These TDP-43 aggregates were not observed in iPSC-MN from healthy subjects, fALS patients, or SMA patients consistent with previous reports from postmortem tissue. These aggregates were also not present in fibroblasts or iPSC of ALS patients (Fig. 2). In order to determine whether aggregates are nuclear or cytoplasmic, patient iPSC-MN motor neurons were co-stained with TDP-43 and a nuclear envelope protein LAMIN-A and imaged the cells by confocal microscopy. Optical sectioning of co-stained cells revealed that TDP-43 aggregates are nuclear (Fig. 4). Custom algorithms were developed to detect and quantify TDP-43 aggregates in confocal images of iPSC-MN cultures immunostained with TDP-43 and ISLET1 (Fig. 5). It was found that TDP-43 aggregates were more frequently present in motor neurons stained with ISLET1 or HB9 as compared to other cells in iPSC-MN cultures (Fig. 3). It was found that 30% of ISLET1+ motor neurons show TDP-43 aggregation as compared to 10% of other cells (ISLET1-HB9 negative) in iPSC-MN cultures. This suggests that TDP-43 aggregates are more likely to form in motor neurons, consistent with the vulnerability of motor neurons in ALS. In order to determine if TDP-43 aggregates are present in motor neuron progenitors, PAX-6 or OLIG-2 positive progenitors within patient iPSC-MN cultures were examined for the presence of aggregates. It was found that progenitors stained with OLIG2 also showed TDP-43 aggregates indicating that in these patient-derived cells, aggregates form early during development. TDP-43 aggregation has not been investigated in human motor neuron progenitors before. While overt differences in neurogenesis between healthy and disease were not observed, it is possible that TDP-43 aggregation in motor neuron progenitors early in development could result in reduced motor neuron pools in ALS patients years before symptoms are present. Existing motor neurons in such individuals would be under increased stress because these motor neurons would have to drive a larger number of muscles and this increased stress could contribute to their vulnerability and loss in adults

leading to development of ALS. Consistent with this notion, athletes have an increased risk of ALS and this may be due to increased stress on the motor neurons in athletes.

[0098] TDP-43 has been shown to be hyperphosphorylated in patient postmortem CNS including at serine 409/410. Patient iPSC-MN cultures were stained using a phospho-specific antibody that recognizes TDP-43 phosphorylated at serine 409/410 residues. It was found that TDP-43 aggregates in patient iPSC-MN are detectable with the phospho-specific antibody indicating that TDP-43 aggregates are phosphorylated in iPSC-MN similar to that observed in patient post-mortem tissue. Patient iPSC-MN were also stained with antibodies raised agains N and C-termini of TDP-43; it was found that both N-term and C-term antibodies stained TDP-43 aggregates suggesting that TDP-43 aggregates contain both N and C-term regions.

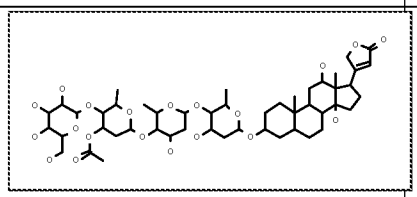
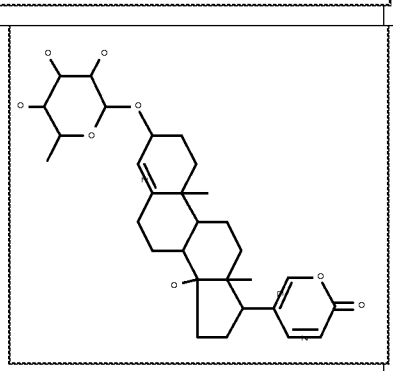
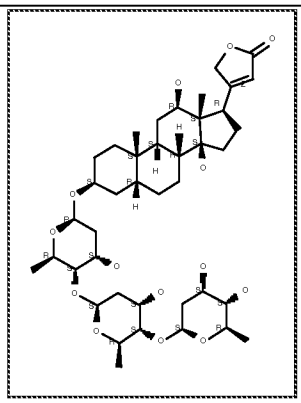
[0099] The data were consistent with the notion that nuclear inclusions may be the first form of pathology in newborn neurons. It is intriguing that, overall, the data indicated that some sporadic ALS patient-derived iPSC lines when differentiated into motor neurons revealed, under basal cell culture conditions, TDP-43 pathology similar to what has been observed in patient postmortem tissue. Thus, it was concluded that patient-derived iPSC-MNs can be used as a cellular model for sporadic ALS and used for discovery of novel therapeutics and identification of molecular mechanisms that lead to TDP-43 aggregation.

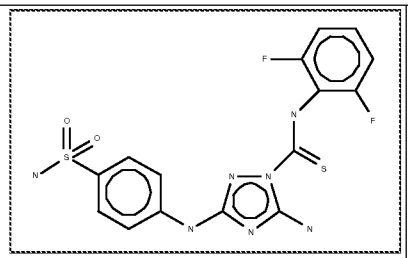
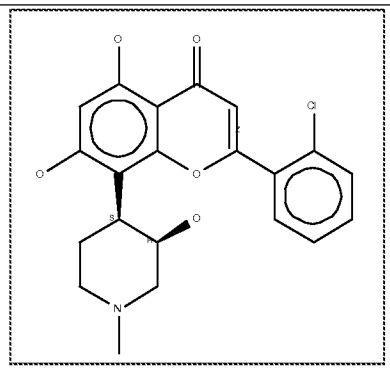
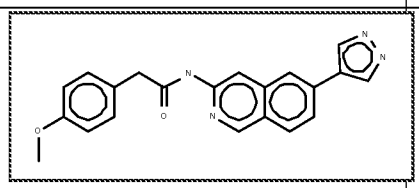
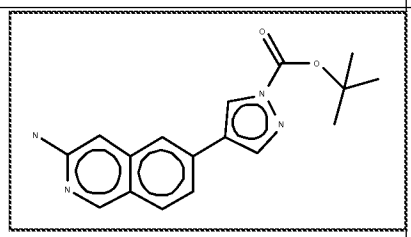
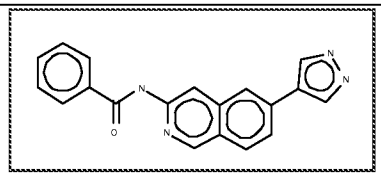
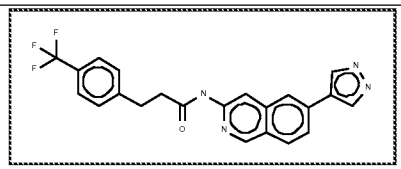
Example 2 Test Screen to Identify Agents that Modulate TDP-43 Aggregation

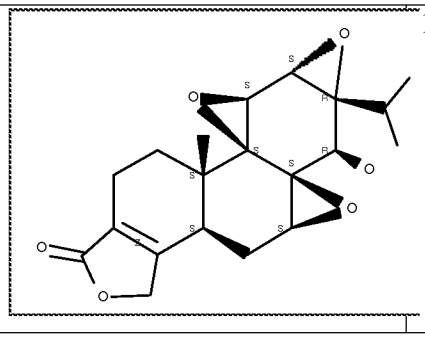
[00100] The TDP-43-aggregate phenotype was developed into an assay for high throughput screening on patient derived iPSC-MN. As a pilot study, screened 56 compounds were screened; it was suspected that these compounds may enhance or suppress TDP-43 aggregation in patient iPSC-MN. The cultures were treated with ten doses of each compound 5 days after plating (day 23) and processed for immunohistochemistry and automated confocal imaging 24 hours and 48 hours after treatment. Analysis of these images revealed that 5 of these compounds reduced the number of cells with aggregates to levels comparable with healthy control iPSC-MN (Fig. 6). Four of these compounds were proteasome inhibitors: MG132, Bortezomib, Epoximicin, Lactacystin, and the other was a JNK/P38 inhibitor having the structure of Formula (III) as described herein.

[00101] In an expanded screen, 1757 bioactive compounds were screened on iPSC-MN from one healthy and one sALS patient in this assay using a custom-built automated screening system. The iPSC-MN cultures were treated with compounds at single concentrations for 48 hours, stained with ISLET1 and TDP-43 antibodies, imaged using confocal microscopy, and the resulting images analyzed for presence of aggregates using custom algorithms. Forty one hits were identified in this primary screen. The hits included 39 compounds that reduced percent cells with aggregates, 2 that increased percent cells with

aggregates, and 1 compound that induced aggregates in a healthy line. As a secondary screen, the hits from the motor neuron screen were screened on iPSC-derived cortical neurons from the same sALS patient in 10 doses for 48 hours. Four classes of compounds were found to reduce TDP-43 aggregates in a dose-dependent manner in sALS patient iPSC-CN. Two of the hits are FDA-approved drugs Digoxin and Lanatoside C and belong to the cardiac glycoside class of compounds and are used to treat heart failure and cardiac arrhythmia. Cardiac glycosides are inhibitors of Na^+/K^+ ATPase pump and can alter Ca^{++} influx into cardiac cells as well as control available ATP levels (Prassas *et al* (2008), *Nat Rev Drug Discov*, 7(11):926-935. Cardiac glycosides have been shown to regulate other cellular signals and possess anti-cancer properties. More importantly cardiac glycosides have been shown to be neuroprotective in two ex vivo brain explant based models of ischaemic stroke and two models for clinical stroke (Prassas *et al supra*). Cardiac glycosides have also been found to inhibit formation of aggresomes in a mutant SOD1-induced cellular model of protein aggregation (Corcoran *et al* (2004), *Curr Biol*, 23;14(6):488-492). Piccioni et al. have reported cardiac glycosides to inhibit polyglutamine-dependent CASP3 activation suggesting a therapeutic role for spinobulbar muscular atrophy (Piccioni *et al* (2004) *Hum Mol Genet*, 13(4):437-446). The findings are consistent with these reports and suggest ionotropic regulation of Na^+/K^+ ATPase pump may be an important regulator TDP-43 aggregation in ALS patient-derived neurons. Although cardiac glycosides do not cross blood brain barrier (BBB), identification of analogs that can penetrate the BBB could lead to novel drugs for ALS and other TDP-43 proteinopathies. These and other hits are listed in Table 2.

Table 2 Overview of Compound Classes and Compounds Identified in TDP-43 Aggregate Modulator Screen					
Compound	Name	structure	EC50 (μM)	Supplier	CAS
Cardiac Glycosides:					
IPC00000441	Lanatoside C		2.717	Sigma-Aldrich L2261	17575-22-3
iPC00004946	Proscillaridin		1.245	Johns Hopkins	
IPC00237745	Digoxin		0.5715	Prestwick	20830-75-5
CDK inhibitors:					

IPC00236556			5.985	EMD 217714	443798-55-8
IPC00236590	Flavopiridol		6.096	enzo	146426-40-6
JNK inhibitors					
IPC0000606	JNK inhibitor		3.732	Incedis Therapeutics	
IPC00236582	IPC0000606 analog			Broadpharm BP-11767	
IPC00236583	IPC0000606 analog		31.12	Nanosyn NSN21363	
IPC00236589	IPC0000606 analog		5.377	Nanosyn NSN21363	
Other					

IPC00000175	Triptolide	 1.96	Sequoia Research SRP02915t	38748-32-2

[00102] These data indicated that sALS patient iPSC-neurons can be used to identify bioactive agents that possess therapeutic potential.

Example 3 Cortical Neurons from Sporadic ALS Patients Exhibit TDP-43 Aggregates

[00103] Human iPSCs were differentiated into cortical neurons (CNs) according to a modified version of Chambers *et al, supra*. In brief, high density iPSCs were cultured on Matrigel™ and differentiated for 10 days in differentiation media (DM) supplemented with 1.5 µM Dorsomorphin and 10 µM SB431542 with daily media changes (DM is a 50:50 mixture of D-MEM/F12: Neurobasal® media supplemented with 5ml/L N2 Supplement (100X), 10 ml/L B-27 without Vitamin A (50X), Glutamax, Penicillin/Streptomycin, 5µg/ml human recombinant Insulin, 100 µM non-essential amino acids and 100 µM β-mercaptoethanol). From Day 11 to day 14, cells were fed with DM media without Dorsomorphin or SB431542. Cells were then fed with DM media supplemented with 0.05 µM retinoic acid from day 15 to 19. At day 20 cultures were dissociated using enzyme-free cell dissociation buffer and replated in DM + 2ng/mL BDNF + 2ng/mL GDNF + 0.05 µM retinoic acid at 1.5 x10^7 cells per poly-L-Lysine (PLL)-Laminin coated 10cm cell culture dish . Cultures were fed every other day from day 21 to day 45 using DM + 2ng/mL BDNF + 2ng/mL GDNF + 0.05 µM retinoic acid. At day 45 cells were either passaged onto new PLL-Laminin coated plates or frozen for subsequent use.

[00104] CNs derived from one of the ALS patients (IPRN.0048) mentioned above in Example 1 were stained for TDP-43 and imaged according to the methods described in Example 1. ALS patient iPSC-CNs from patient IPRN.0048 had intranuclear TDP-43 aggregates (Fig. 7A) similar to those found in motor neurons from this patient. These data indicate that iPSC-derived neurons of two different lineages that are known to degenerate in ALS recapitulate TDP-43 aggregation in vitro and that these neurons can be used to model TDP-43 pathology.

[00105] While intranuclear TDP-43 aggregates have been more commonly reported in FTD with GRN mutations and fALS with VCP mutations, few data have been reported on the presence of intranuclear aggregates in

sporadic ALS patient neurons. In order to determine if intranuclear TDP-43 aggregates were present in patients from whom iPSC-MNs and iPSC-CNs were generated, postmortem brain and spinal cord tissue from one of the patients with TDP-43 phenotype in iPSC-MN (patient IPRN.0360) were analyzed for the presence of TDP-43 pathology. Anterior horn neurons of the spinal cord as well as cortical neurons from this patient had round intranuclear TDP-43 aggregates that stained with higher intensity (Fig. 7B) and were morphologically similar to those found in the patient's iPSC-MNs. These aggregates were present in cervical, lumbar, and thoracic spinal cord as well as frontal lobe neurons (Fig. 7B, right panel,).

[00106] Some anterior horn neurons also showed other forms of TDP-43 pathology such as cytoplasmic staining (Fig. 7B, arrowhead in left panel). These data indicate that intranuclear TDP-43 aggregates occur in sporadic ALS patients and co-exist with other forms of TDP-43 pathology. These data indicate that intranuclear TDP-43 pathology present in an sALS patient's postmortem CNS can be recapitulated in cultured iPSC-MNs from the same patient.

[00107] In order to determine if any of the three sALS patients might have mutations that could result in TDP-43 pathology in their iPSC-MN, several genes that are known to be mutated in fALS and have been associated with TDP-43 pathology were sequenced. No mutations in Granulin (GRN), TDP-43, Valsolin-containing protein (VCP), vesicle-associated membrane protein B (VAPB), or expansion in hexanucleotide repeats in c9ORF72, or CAG repeats in Ataxin2 (ATXN2) were found in these three patients suggesting an uncharacterized genetic or epigenetic alteration as the initiator of TDP-43 aggregate formation in these sALS patients.

[00108] While preferred embodiments of the present invention have been shown and described herein, such embodiments are provided by way of example only. Numerous variations, changes, and substitutions are possible without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a JNK or a MAPK inhibitor.

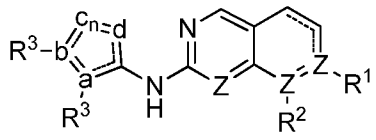
2. The method of claim 1, wherein the TDP-43 proteinopathy is ALS or FTD.

3. The method of claim 1, wherein the TDP-43 proteinopathy is ALS.

4. The method of claim 1, comprising administering to the subject a therapeutically effective amount of a JNK inhibitor.

5. The method of claim 1, comprising administering to the subject a therapeutically effective amount of a MAPK inhibitor.

6. A method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a compound having the structure of Formula (I):



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R^1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R^2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

each R^3 is not present or is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, and SO_2R^x;

each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

each Z is selected from C and N;

a is selected from C, N, O, and S;

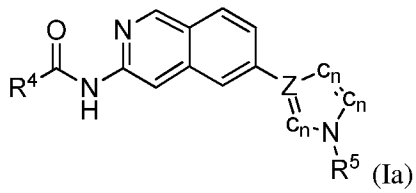
b is selected from C, N, O, and S;

c is selected from C, N, O, and S;

n is zero, one, or 2; and

d is selected from C, N, O, and S, when n is one or 2; or d is selected from O and S, when n is zero.

7. The method of claim 6, wherein the structure of Formula (I) has the structure of Formula (Ia):



(Ia)

wherein:

R^4 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, and OR^x;

R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

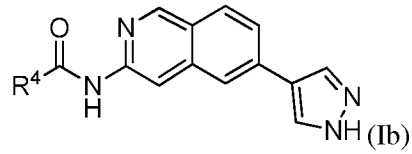
Z is selected from C and N;

c is selected from C, N, O, and S;

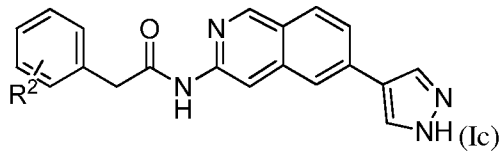
n is zero, one, or 2; and

R^5 is not present or is selected from hydrogen, alkyl, aryl, and heterocyclic.

8. The method of claim 6, wherein the structure of Formula (I) has the structure of Formula (Ib) or Formula (Ic):



(Ib)



(Ic)

wherein:

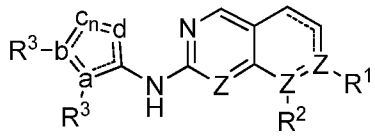
R^4 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, and OR^x;

R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

R^2 is selected from alkyl, substituted alkyl, alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino. aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclic, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-aryl, and $-SO_2$-heteroaryl.

9. A method for reducing the number or size of TDP-43 protein aggregates in a population of cells, comprising contacting a population of cells having TDP-43 protein aggregates with a JNK inhibitor or a compound having the structure of Formula (I):



(I)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R^1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R^2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

each R^3 is not present or is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, and SO_2R^x;

each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

each Z is selected from C and N;

a is selected from C, N, O, and S;

b is selected from C, N, O, and S;

c is selected from C, N, O, and S;

n is zero, one, or 2; and

d is selected from C, N, O, and S, when n is one or 2; or d is selected from O and S, when n is zero.

10. A method for a identifying an agent that modulates TDP-43 aggregation in human neurons or human neural progenitors, comprising determining in a population of cells comprising human neurons or human neural progenitors the level of TDP-43 aggregates in the presence or absence of a test agent, wherein a plurality of the human neurons or human neural progenitors contain one or more TDP-43 aggregates in the absence of the test agent; and identifying the test agent as an agent that modulates TDP-43 aggregation in human neurons or human neural progenitors if the TDP-43 aggregation in the plurality of human neurons in the presence of the test agent is different from the TDP-43 aggregation in the plurality of human neurons in the absence of the test agent.

11. The method of claim 10, comprising identifying a test agent that reduces the number of TDP-43 aggregates in the plurality of human neurons or human neural progenitors.

12. The method of claim 10, wherein the plurality of human neurons or human neural progenitors comprises Islet-positive cells.

13. The method of claim 10, wherein the plurality of human neurons comprises HB9-positive cells.

14. The method of claim 10, wherein the plurality of human neurons or human neural progenitors comprises motor neurons.

15. The method of claim 10, wherein the population of cells is derived from a subject suffering from ALS or FTD.

16. The method of claim 15, wherein the subject is suffering from ALS.

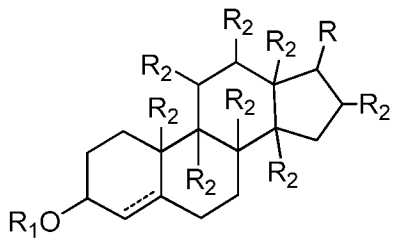
17. The method of claim 15, wherein the ALS is a sporadic form of ALS.

18. An isolated population of human cells comprising viable neurons or neural progenitors, wherein: (i) a plurality of the viable neurons or neural progenitors comprise an endogenous TDP-43 aggregate; (ii) the isolated population is derived from a subject suffering from ALS or FTD.

19. A human induced pluripotent stem cell line from a subject suffering from sporadic ALS, wherein a plurality of motor neurons or neural progenitors obtained by differentiating the human induced pluripotent stem cell line comprise a TDP-43 aggregate.

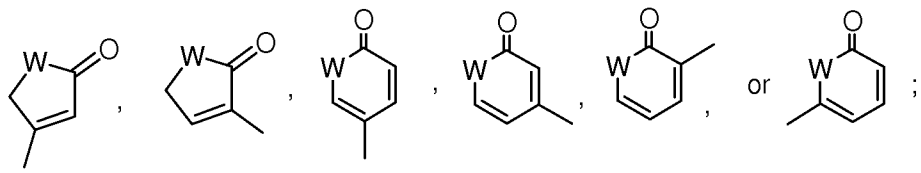
20. The human induced pluripotent stem cell line of claim 19, wherein the ALS is a sporadic form of ALS.

21. A method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a compound having the structure of Formula (XIV):



(XIV)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R is  ;

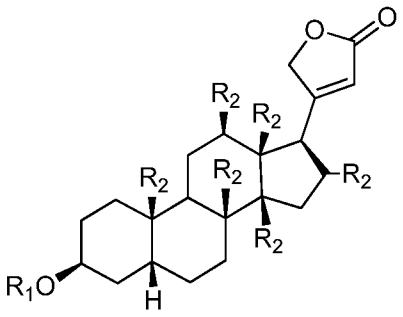
R_1 is selected from a sugar species and hydrogen;

R_2 is independently selected from OH, alkyl, hydrogen, alkyl, OH, and CHO; and

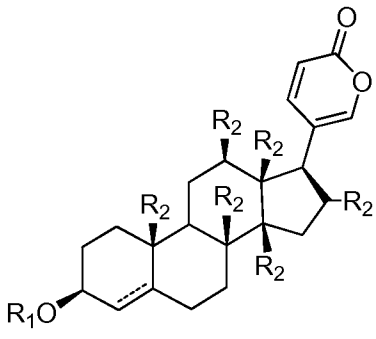
W is selected from O and NR_3; and

R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

22. The method of claim 21, wherein the structure of Formula (XIV) has the structure of Formula (XIVa) or (XIVb):



(XIVa)



(XIVb)

wherein:

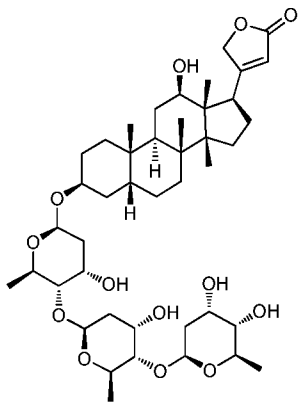
R_1 is selected from a sugar species and hydrogen;

R_2 is independently selected from OH, alkyl, hydrogen, alkyl, OH, and CHO; and

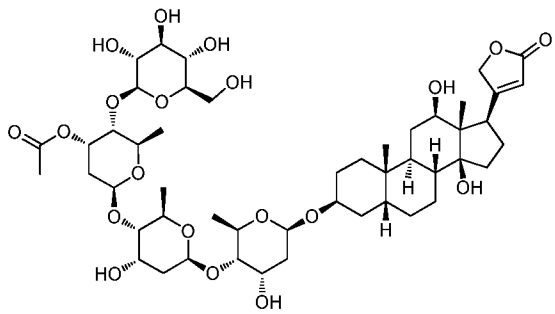
W is selected from O and NR_3; and

R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

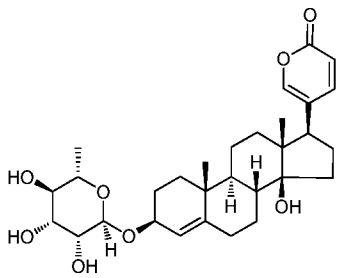
23. The method of claim 21, wherein the structure of Formula (XIV) has the structure of any of Formulas (XIVc) to (XIVg):



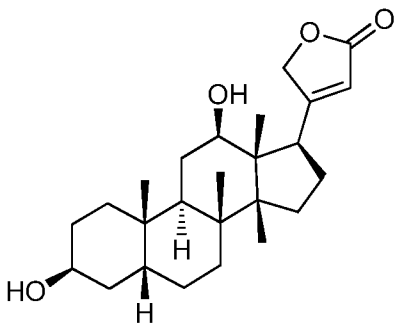
(XIVc)



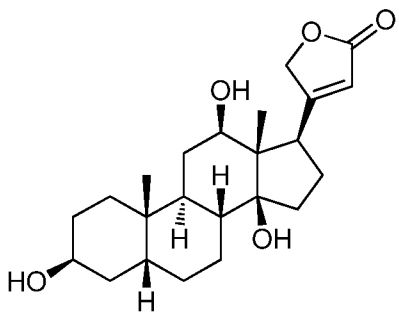
(XIVd)



(XIVe)

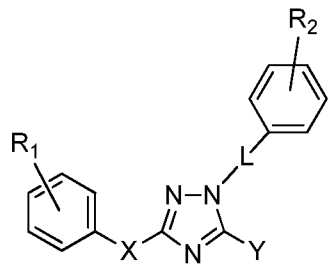


(XIVf)



(XIVg)

24. A method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a compound having the structure of Formula (XV):



(XV)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R_1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R_2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

L is not present or is selected from $-C(O)NR_4-$, $-C(O)NR_4$-alkyl-, $-C(O)-$, $-NR_4-$;

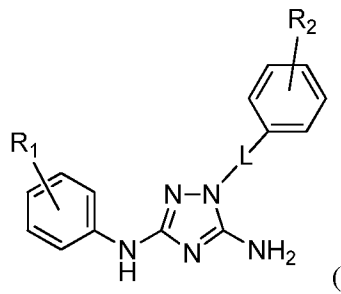
R_4 is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

X is selected from O and NR_3;

R_3 is selected from alkyl, substituted alkyl, aryl, and substituted aryl; and

Y is selected from OH and NH_2.

25. The method of claim 24, wherein the structure of Formula (XV) has the structure of Formula (XVa):



(XVa)

wherein:

R_1 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

R_2 is selected from hydrogen, alkyl, aryl, heterocyclic, NR^xR^y, OR^x, SR^x, SOR^x, SO_2R^x, halogen, and CN;

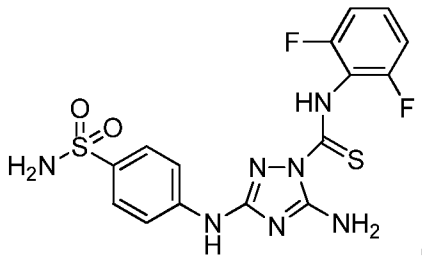
each R^x is selected from alkyl, substituted alkyl, aryl, substituted aryl, amino, and substituted amino;

each R^y is selected from alkyl, substituted alkyl, aryl, and substituted aryl;

L is not present or is selected from $-C(O)NR_4-$, $-C(O)NR_4$-alkyl-, $-C(O)-$, $-NR_4-$; and

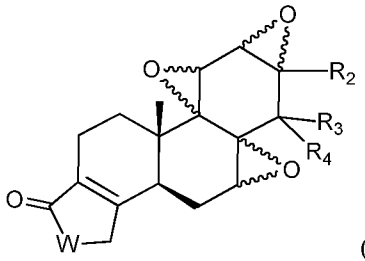
R_4 is selected from alkyl, substituted alkyl, aryl, and substituted aryl.

26. The method of claim 24, wherein the structure of Formula (XV) has the structure of Formula (XVb):



(XVb)

27. A method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a therapeutically effective amount of a compound having the structure of Formula (XVI):



(XVI)

or a pharmaceutically acceptable salt or solvate thereof, wherein:

R^2 is selected from hydrogen, alkyl, alkylhydroxy, hydroxyalkyl, aryl, and heterocyclic;

R^3 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic;

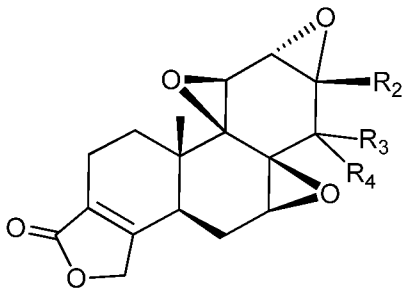
R^4 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic; or

R^3R^4 taken together is =O or NR^1; and

W is selected from O and NR^1;

wherein each R^1 is independently selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic.

28. The method of claim 27, wherein the structure of Formula (XVI) has the structure of Formula (XVIa):



(XVIa)

wherein:

R^2 is selected from hydrogen, alkyl, alkylhydroxy, hydroxyalkyl, aryl, and heterocyclic;

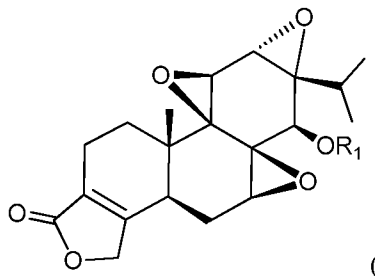
R^3 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic;

R^4 is selected from hydrogen, alkyl, alkoxy, amino, aryl, and heterocyclic; or

R^3R^4 taken together is =O or NR^1; and

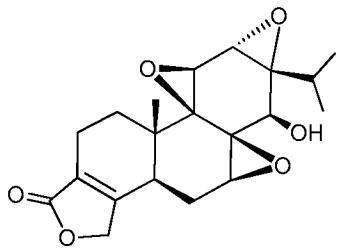
R^1 is selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic.

29. The method of claim 27, wherein the structure of Formula (XVI) has the structure of Formula (XVIb) or Formula (XVIc):



(XVIb)

wherein R^1 is selected from hydrogen, alkyl, ester, urea, carbamate, aryl, and heterocyclic;



(XVIc)

30. A method for treating a subject suffering from a TDP-43 proteinopathy, comprising administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of a proteasome inhibitor, a Topoisomerase I inhibitor, a Topoisomerase II inhibitor, a HSP-90 inhibitor, a 5-HT antagonist, a CDK inhibitor, or a transcription inhibitor.

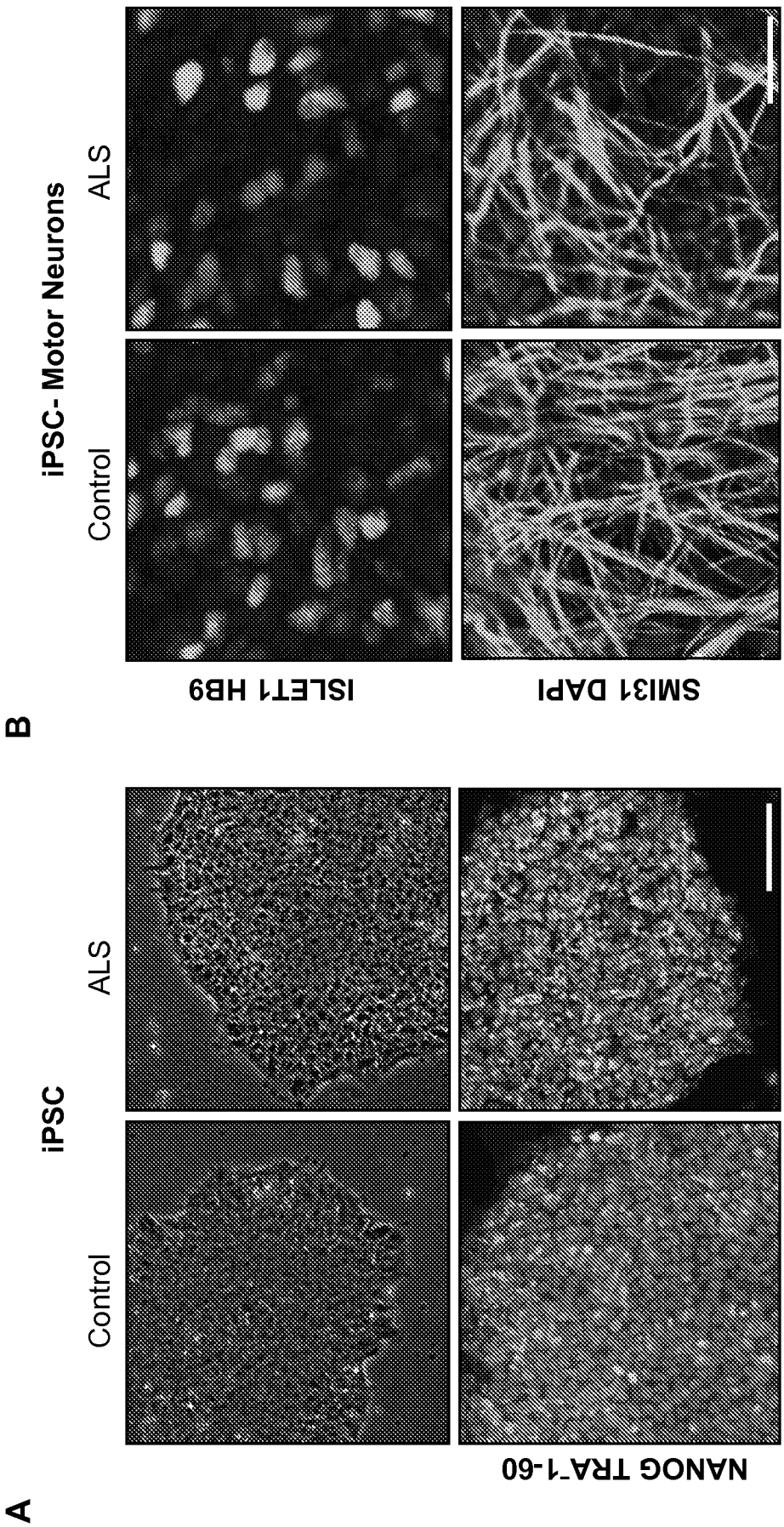

A
B
iPSC
iPSC- Motor Neurons
Control
ALS
NANOG TRA-1-60
ISLET1 HB9
SMI31 DAPI

FIG. 1

A

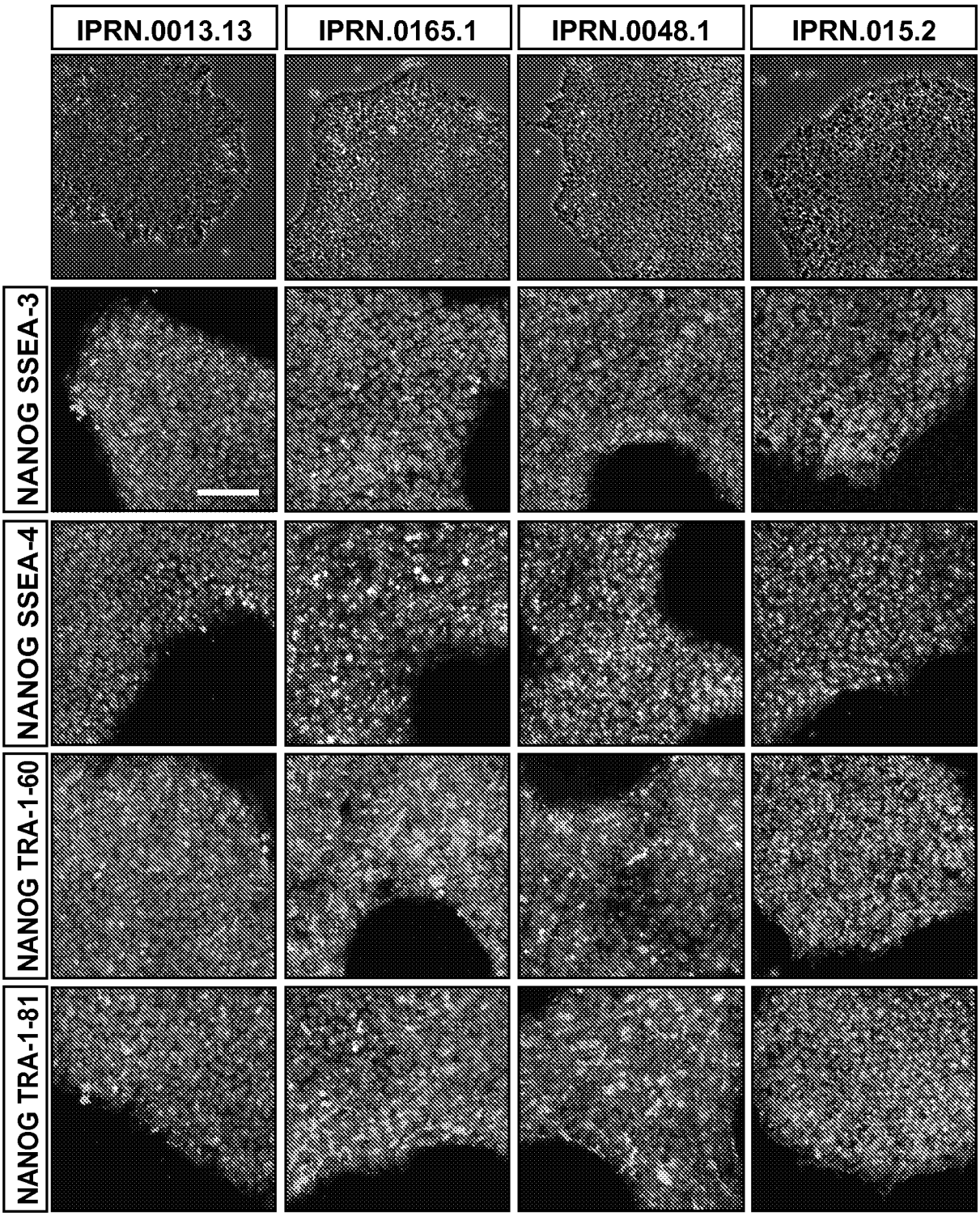

IPRN.0013.13
IPRN.0165.1
IPRN.0048.1
IPRN.015.2
NANOG SSEA-3
NANOG SSEA-4
NANOG TRA-1-60
NANOG TRA-1-81

FIG. 2

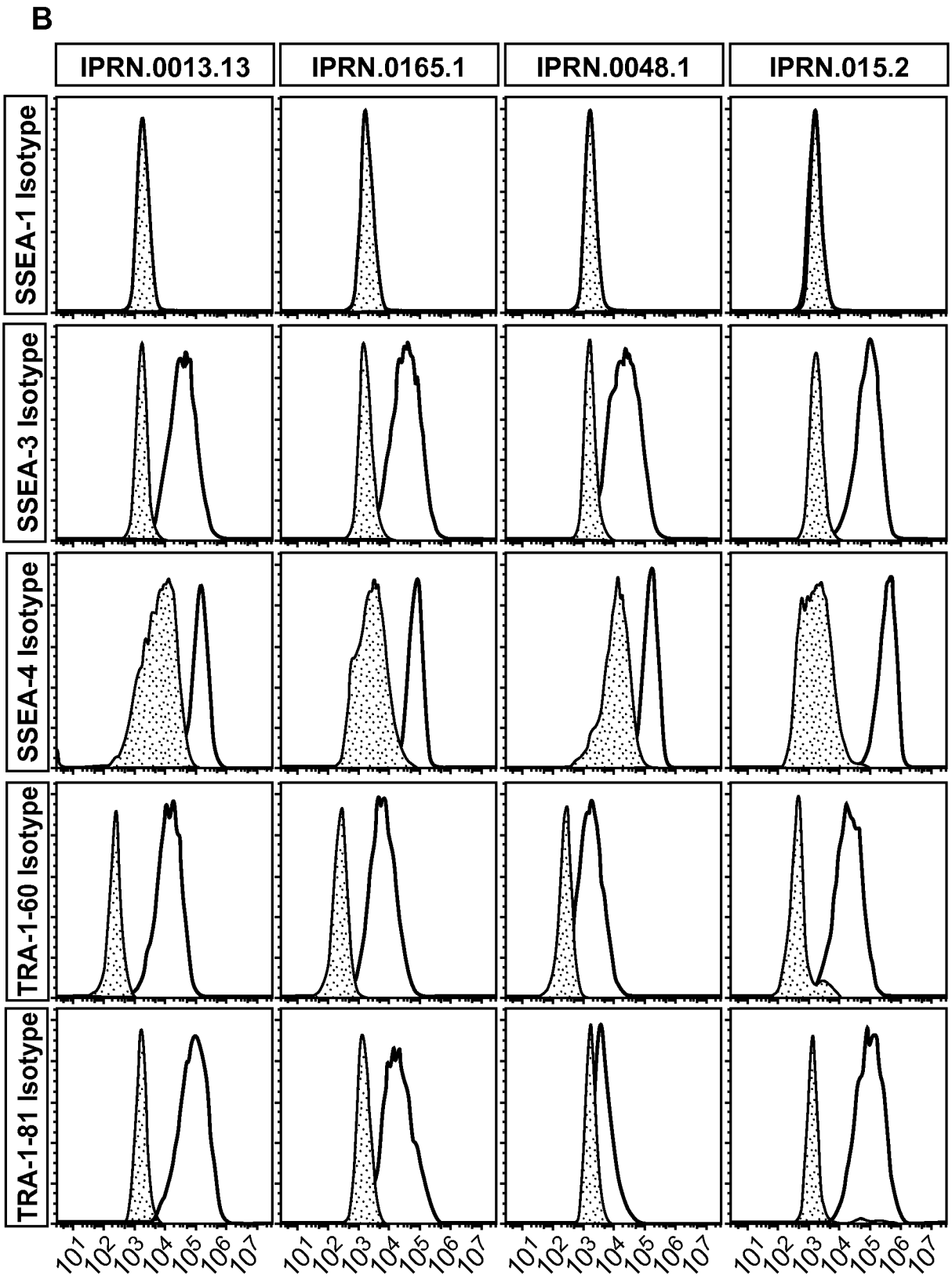

B
IPRN.0013.13
IPRN.0165.1
IPRN.0048.1
IPRN.015.2
SSEA-1 Isotype
SSEA-3 Isotype
SSEA-4 Isotype
TRA-1-60 Isotype
TRA-1-81 Isotype
10^1 10^2 10^3 10^4 10^5 10^6 10^7

FIG. 2 (Cont.)

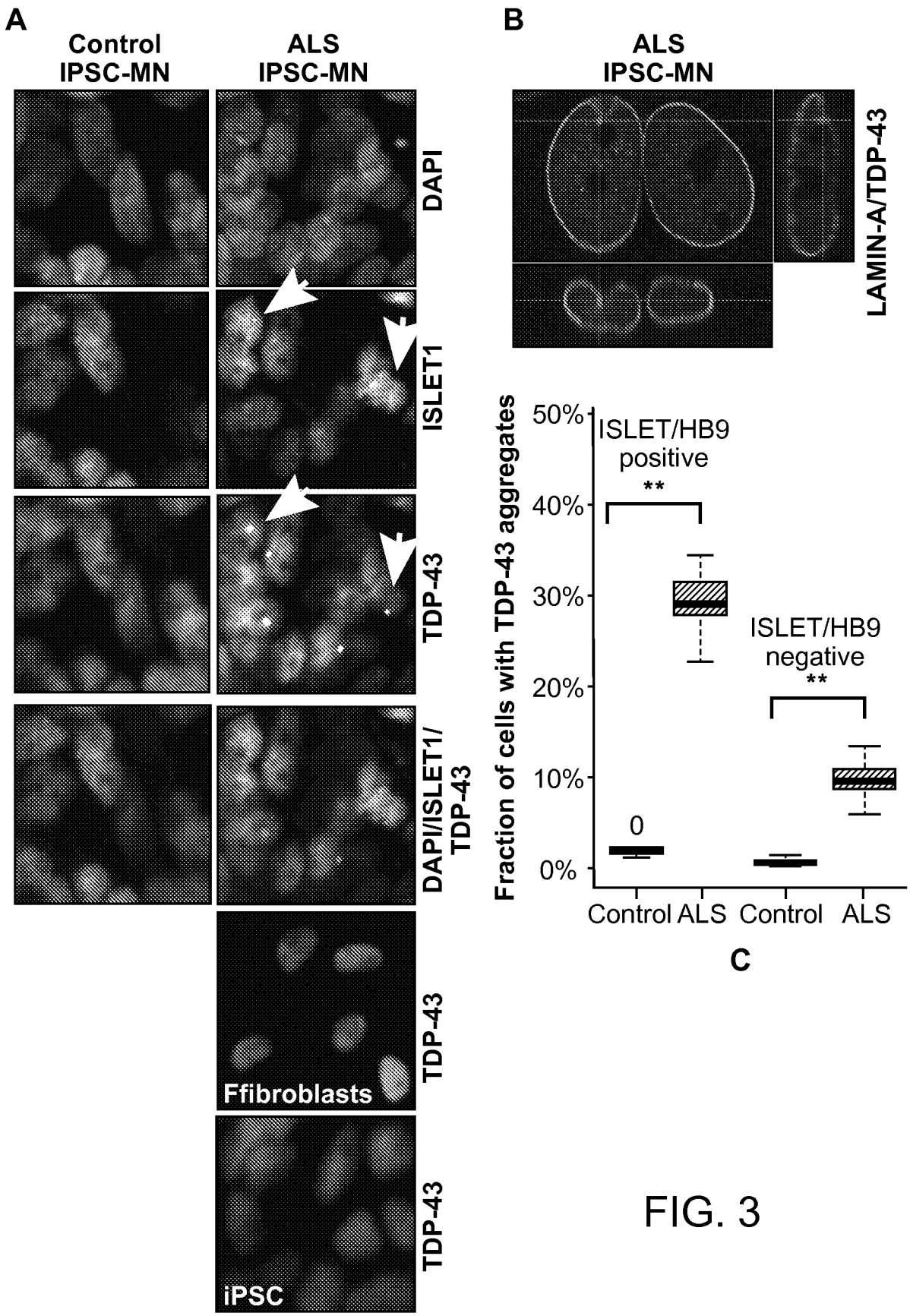
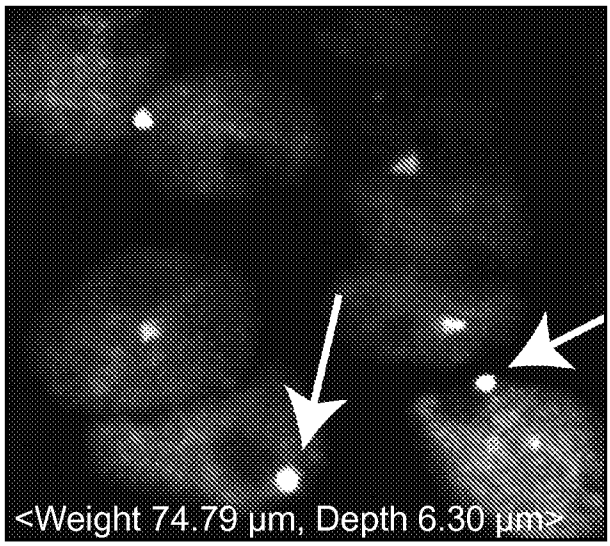

A
Control IPSC-MN
ALS IPSC-MN
DAPI
ISLET1
TDP-43
DAPI/ISLET1/ TDP-43
TDP-43
Ffibroblasts
TDP-43
iPSC
B
ALS IPSC-MN
LAMIN-A/TDP-43
Fraction of cells with TDP-43 aggregates
50%
40%
30%
20%
10%
0%
ISLET/HB9 positive
**
ISLET/HB9 negative
**
0
Control ALS Control ALS
C

FIG. 3

IPSC-MN from ALS patient: IPRN.0048.1

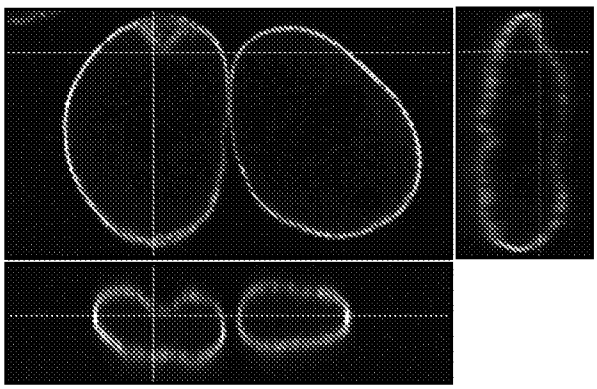
A

TDP-43 stain in ALS patient iPSC-MN

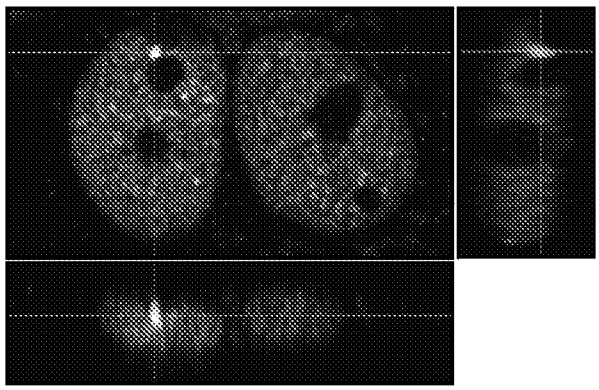


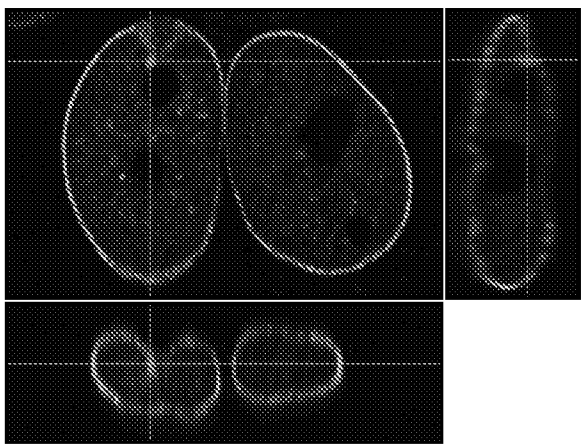
B

LAMIN-A



TDP-43





LAMIN-A/TDP-43 merge

FIG. 4

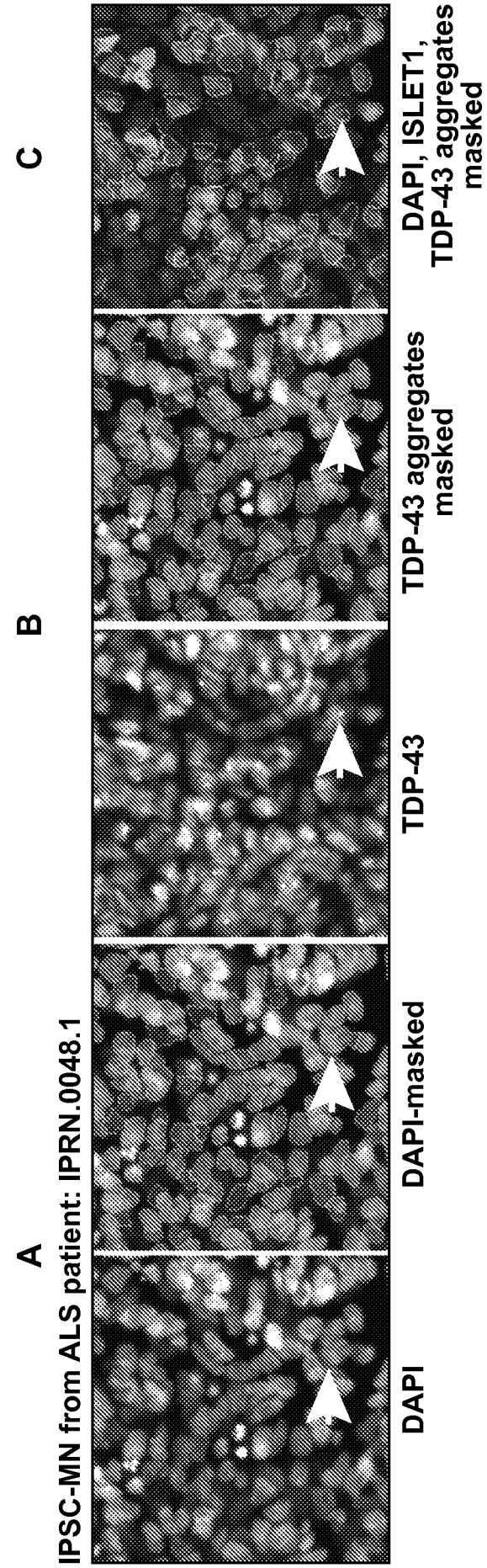

A
B
C
IPSC-MN from ALS patient: IPRN.0048.1
DAPI
DAPI-masked
TDP-43
TDP-43 aggregates masked
DAPI, ISLET1, TDP-43 aggregates masked

FIG. 5

A

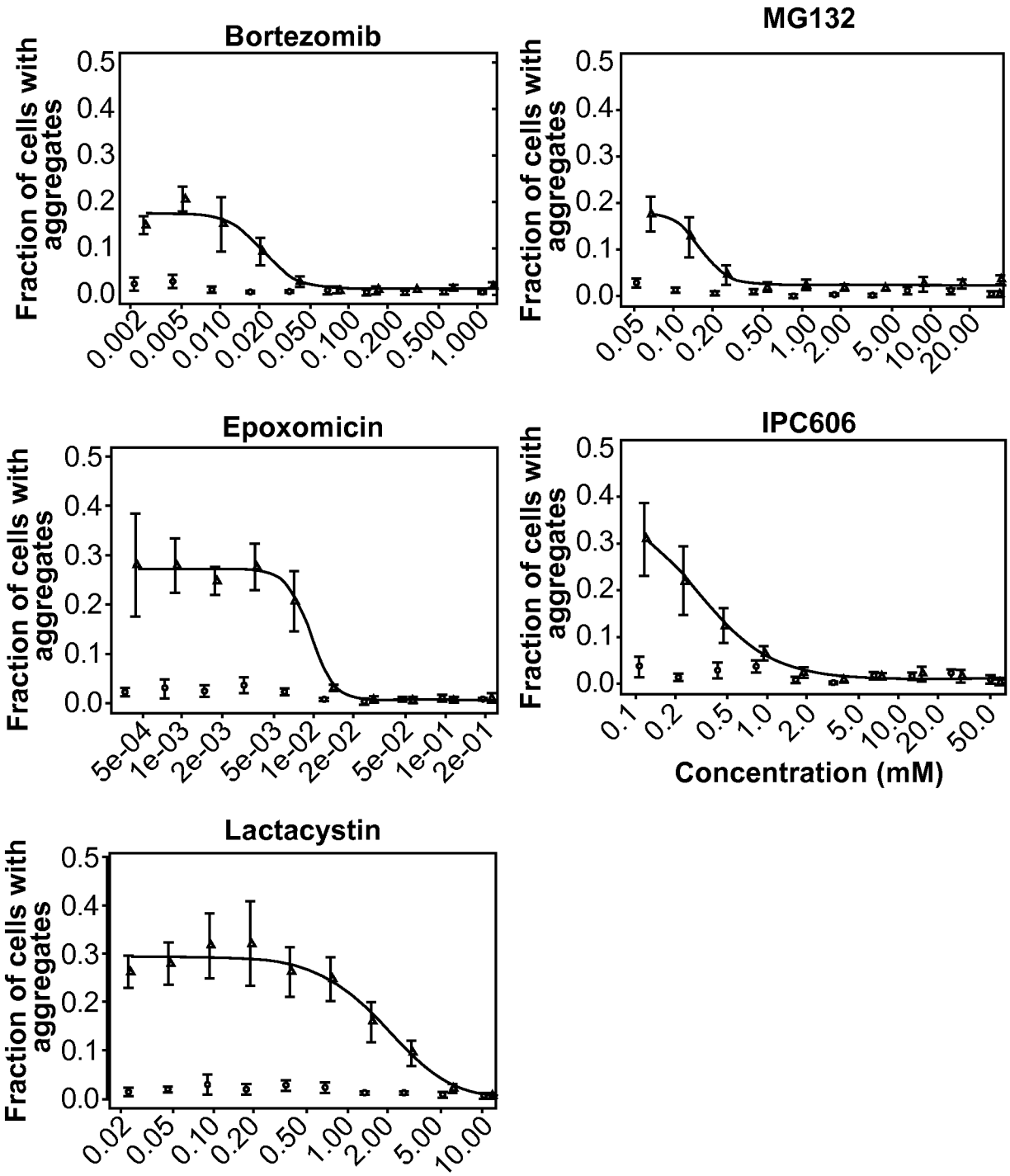

Bortezomib
MG132
Epoxomicin
IPC606
Lactacystin
Fraction of cells with aggregates
0.0
0.1
0.2
0.3
0.4
0.5
0.002
0.005
0.010
0.020
0.050
0.100
0.200
0.500
1.000
0.05
0.10
0.20
0.50
1.00
2.00
5.00
10.00
20.00
5e-04
1e-03
2e-03
5e-03
1e-02
2e-02
5e-02
1e-01
2e-01
0.1
0.2
0.5
1.0
2.0
5.0
10.0
20.0
50.0
Concentration (mM)
0.02
0.05
0.10
0.20
0.50
1.00
2.00
5.00
10.00

FIG. 6

B

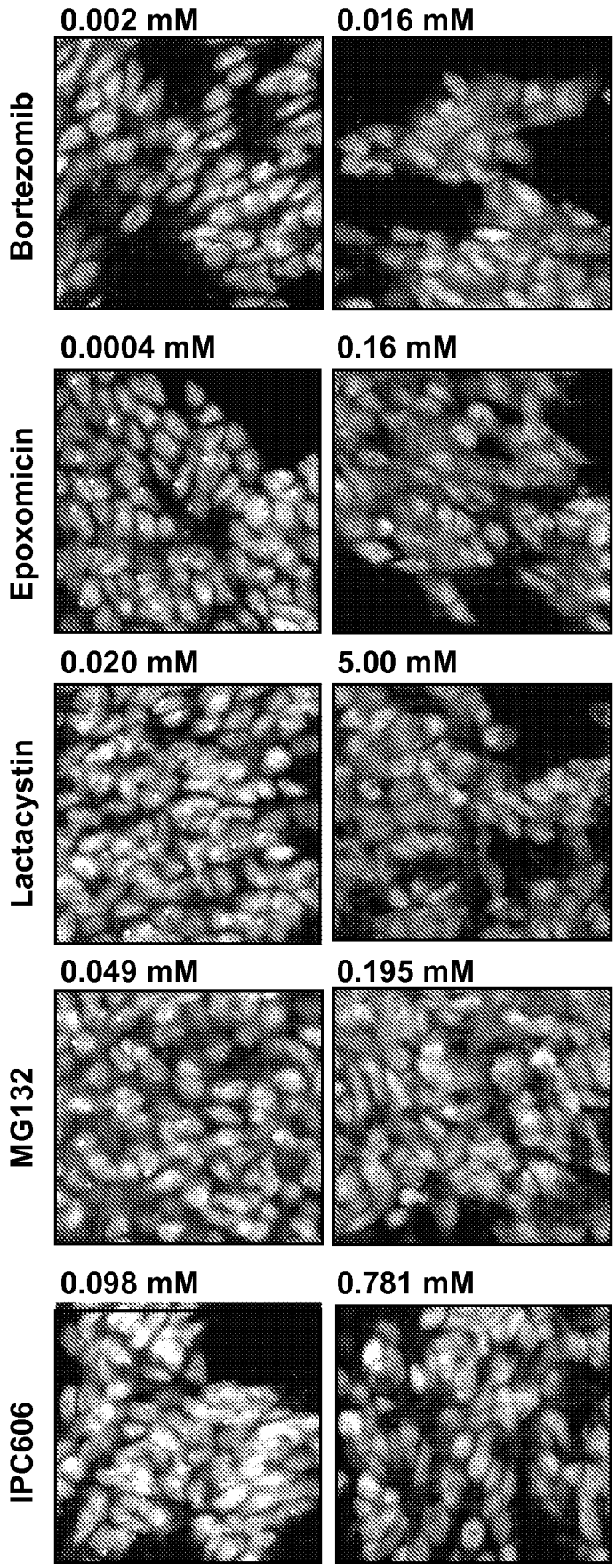

0.002 mM
0.016 mM
Bortezomib
0.0004 mM
0.16 mM
Epoxomicin
0.020 mM
5.00 mM
Lactacystin
0.049 mM
0.195 mM
MG132
0.098 mM
0.781 mM
IPC606

FIG. 6 (Cont.)

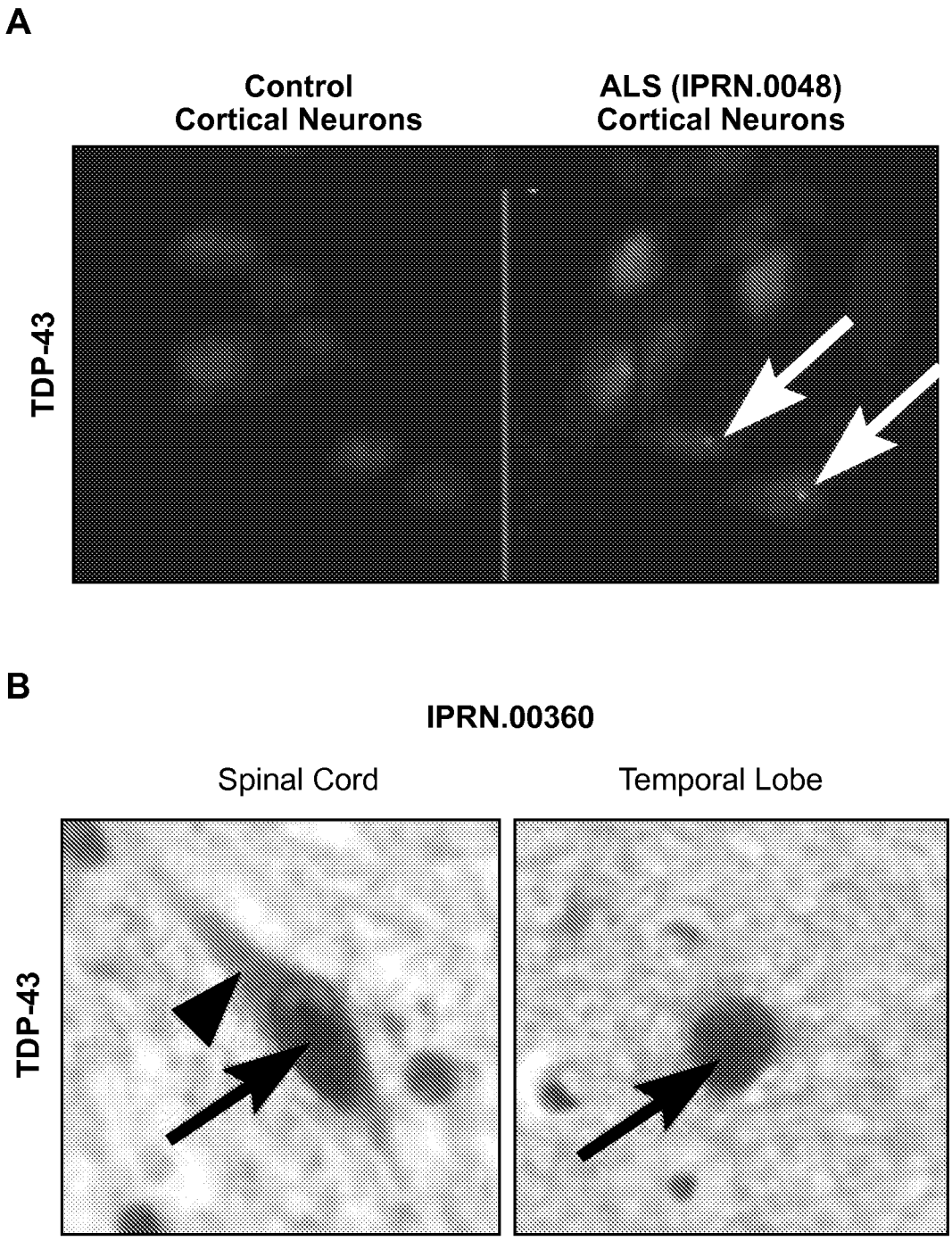

A
Control
Cortical Neurons
ALS (IPRN.0048)
Cortical Neurons
TDP-43
B
IPRN.00360
Spinal Cord
Temporal Lobe
TDP-43

FIG. 7

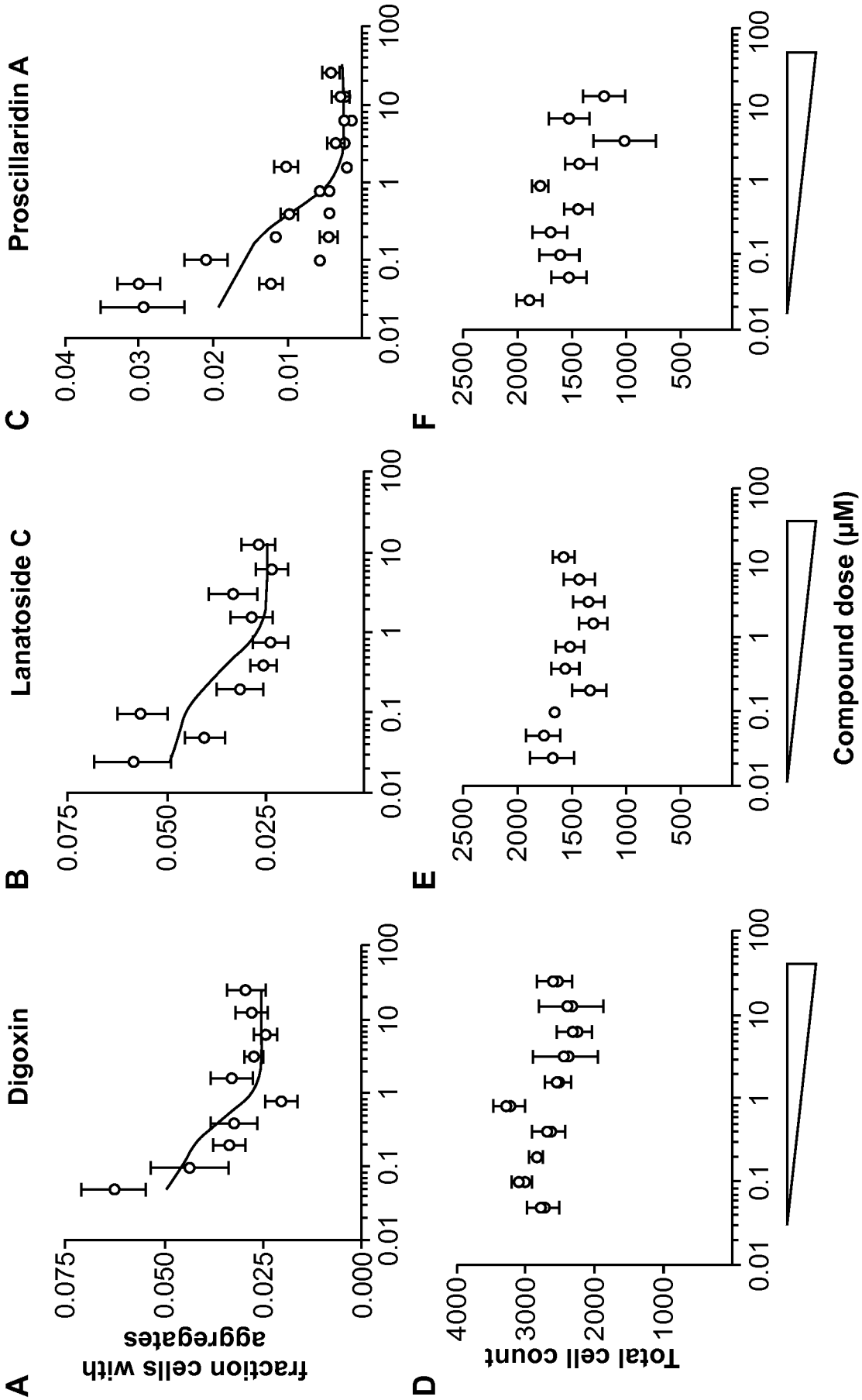

A
Digoxin
fraction cells with aggregates
0.075
0.050
0.025
0.000
0.01
0.1
1
10
100
B
Lanatoside C
0.075
0.050
0.025
C
Proscillaridin A
0.04
0.03
0.02
0.01
D
Total cell count
4000
3000
2000
1000
E
2500
2000
1500
1000
500
F
Compound dose (μM)

FIG. 8