Claims

1. A kit for treating a protein degradation disorder in a subject, the kit comprising:

an aggresome inhibitor that selectively inhibits HDAC6 or a pharmaceutically acceptable ester or salt ~~esters, salts, and prodrugs~~ thereof; and

instructions for using the aggresome inhibitor in combination with a proteasome inhibitor for treating the protein degradation disorder in the subject.

2. The kit of claim 2, wherein the aggresome inhibitor is present as a pharmaceutical composition comprising a therapeutically effective amount of the aggresome inhibitor and a pharmaceutically acceptable carrier.

3. A composition comprising a therapeutically effective amount of an aggresome inhibitor that selectively inhibits HDAC6 and a pharmaceutically acceptable carrier or diluent, wherein the composition is formulated for treating a subject suffering from or susceptible to a protein degradation disorder and is packaged with instructions to treat the subject with the aggresome inhibitor in combination with a proteasome inhibitor.

4. The kit or composition of claim 1, 2 or 3, wherein the protein degradation disorder is a cellular proliferation disorder or a protein deposition disorder.

5. The kit or composition of claim 4, wherein the cellular proliferation disorder is cancer.

6. The kit or composition of claim 5, wherein the cancer is one or more of multiple myeloma, leukemia, lymphoma, breast cancer, lung cancer and liver cancer.

7. The kit or composition of claim 5, wherein the cancer is multiple myeloma.

8. The kit or composition of claim 4, wherein the protein deposition disorder is Wilson's disease, spinocerebellar ataxia, prion disease, Parkinson's disease, Huntington's disease, Familial amytrophic lateral sclerosis, amyloidosis, Alzheimer's disease, Alexander's disease, alcoholic liver disease, cystic fibrosis, Pick's Disease or Lewy body dementia.

9. The kit or composition of any one of claims 1 to 8, wherein the protein degradation disorder is an inflammatory disease.

~~10. A method of selecting a subject with a protein degradation disorder for a treatment, comprising:~~

~~determining one or more pre-treatment phenotypes; and~~

~~determining the one or more phenotypes after the subject has been administered a therapeutically effective amount of an aggresome inhibitor that selectively inhibits HDAC6 in combination with a proteasome inhibitor;~~

~~wherein the modulation of the one or more phenotype is an indication that the disorder is likely to have a favorable clinical response to treatment with the aggresome inhibitor in combination with a proteasome inhibitor.~~

~~11~~10. A method of inhibiting protein degradation in a cell comprising contacting the cell *in vitro* with an aggresome inhibitor that selectively inhibits HDAC6 in combination with a proteasome inhibitor.

~~12. A method of assessing the efficacy of a protein degradation disorder treatment in a subject, comprising:~~

~~determining one or more pre-treatment phenotypes; and~~

~~determining the one or more phenotypes after the subject has been treated with a therapeutically effective amount of an aggresome inhibitor that selectively inhibits HDAC6 in combination with a proteasome inhibitor;~~

~~wherein the modulation of the one or more phenotypes indicates efficacy of the treatment with the aggresome inhibitor in combination with a proteasome inhibitor.~~

~~13~~11. The kit, composition or method of any one of claims 1 to ~~12~~10, wherein the proteasome inhibitor is selected from the group consisting of bortezomib, MG132, sapojargon, and NPI-0052.

~~14~~12. The kit, composition or method of any one of claims 1 to ~~12~~10, wherein the aggresome inhibitor is tubacin.

~~15~~13. The kit, composition or method of any one of claims 1 to ~~13~~12, wherein the aggresome inhibitor comprises a metal binding moiety.

~~16~~14. The kit, composition or method of claim ~~15~~13, wherein the metal binding moiety is a zinc-binding moiety.

~~17~~15. The kit, composition or method of claim ~~15~~14, wherein the zinc-binding moiety is a hydroxamate moiety.

16~~18~~. The kit composition or method of claim ~~16~~15, wherein the hydoxamate moiety comprises a cinnamic hydroxamate, an O-ether of hydroxamate or a cyclic hydroxamate.

~~19~~17. The kit, composition or method of claim ~~16~~15, wherein the hydroxamate moiety comprises an alkylene chain substituted with a terminal hydroxamate group.

~~20~~18. The kit, composition or method of claim ~~16~~15, wherein the hydroxamate moiety comprises a phenyl group substituted with an amido group bearing an alkylene moiety, in which the alkylene chain has between four and eight carbon atoms, and the alkylene chain is substituted with a terminal hydroxamate group.

~~21~~19. The kit, composition or method of any one of claims ~~16~~ 14 to ~~20~~18, wherein the aggresome inhibitor includes a linker arm or other spacing moiety between the zinc-binding moiety and a target binding portion of the aggresome inhibitor responsible for binding to its biological target.

~~22~~20. The kit, composition or method of claim ~~21~~19, wherein, when the target binding portion of the aggresome inhibitor is bound to its biological target, the linker arm or other spacing moiety orientates the zinc-binding moiety into a position favorable for target inhibition.

~~23~~21. The kit, composition or method of any one of claims 1 to ~~13~~11, wherein, the aggresome inhibitor inhibits the C-terminal aceylation activity of HDAC6, thereby inhibiting aggresome mediated protein degradation.

~~24~~22. The kit, composition or method of any one of claims 1 to ~~13~~11, wherein the aggresome inhibitor is a peptide derived from HDAC6, dyenin, an N-terminal peptide of HDAC6 or C-terminal peptide of HDAC6.

~~25~~23. The kit, composition or method of claim ~~24~~22, wherein the C-terminal HDAC6 peptide is sufficient to modulate a phenotype of a cell.

~~26. The method of claim 10 or 12, wherein the phenotype is biological or clinical sequelae in response to a particular treatment or compound, anemia, thrombocytopenia, neutropenia, osteolytic lesions, bone pain, immunodeficiency, renal insufficiency, hypercalcemia, aneuploidy of mature plasma cells, percentage of malignant cells, acetylation state of tubulin, apoptosis of mature plasma cells, level of aggresomes in mature plasma cells, HDAC6 ubiquitination in mature plasma cells, HDAC6 association with dynein in mature plasma cells, cellular levels of ubiquintinated proteins in mature plasma cells, level of caspase-8 in mature plasma cells, level of PARP in mature plasma cells, thymidine uptake in mature plasma cells, dilated ER cisternae, aggregation of mature plasma cells, deposits of immunoglobulins in mature plasma cells, acetylation state of non-histone proteins, global ubiquitination state of the cellular proteins, state of cell cycle regulation, necrosis, markers of apoptosis, apoptosis state, Russell body formation, Cystic Fibrosis transmembrane protein receptor state, and modulation of cellular protein deposits, or global acetylation state of cellular or extracellular proteins.~~

27. The method of claim 26, wherein a decrease in one or more of level of aggresomes, thrombocytopenia, neutropenia, osteolytic lesions, bone pain, immunodeficiency, renal insufficiency, hypercalcemia, aneuploidy of mature plasma cells, percentage of malignant cells, thymidine uptake in mature plasma cells, level of full length caspase-8 in mature plasma cells, level of full length PARP in mature plasma cells, or aggregation of mature plasma cells, indicates that the treatment is efficacious.

28. The method of claim 26, wherein an increase in acetylation state of tubulin, HDAC6 ubiquitination in mature plasma cells, level of cleaved form of caspase-8, level of cleaved form of PARP, necrosis, acetylation state of non-histone proteins, cellular ubiquitination levels, apoptosis, markers of apoptosis, cell cycle deregulation, or deposits of immunoglobulins in mature plasma cells indicates that the treatment is efficacious.

29. The method of claim 10 or 12, further comprising obtaining a biological sample from a subject.

30. The method of claim 10 or 12, further comprising determining the subject's phenotype after a second administration of the aggresome inhibitor that selectively inhibits HDAC6 and/or the proteasome inhibitor.

31. The method of claim 10 or 12, further comprising obtaining a second biological sample from the subject.

32. The method of claim 10 or 12, further comprising monitoring the treatment or progress of the cell or subject.

33. The method of claim 10 or 12, further comprising obtaining the aggresome inhibitor and/or the proteasome inhibitor.

34. The method of claim 10 or 12, wherein the subject is co-administered one or more of a chemotherapeutic agent, radiation agent, hormonal agent, biological agent or an anti-inflammatory agent to the subject.

35. The method of claim 34, wherein the chemotherapeutic agent is tamoxifen, trastuzamab, raloxifene, doxorubicin, fluorouracil/5-FU, pamidronate disodium, anastrozole, exemestane, cyclophosphamide, epirubicin, letrozole, toremifene, fulvestrant, fluoxymester-one, trastuzumab, methotrexate, megastrol acetate, docetaxel, paclitaxel, testolactone, aziridine, vinblastine, capecitabine, goselerin acetate, zoledronic acid, taxol, vinblastine, or vincristine.

36. The method of claim 10 or 12, further comprising comparing one or more of the pre-treatment or post-treatment phenotypes to a standard phenotype.

37. The method of claim 36, wherein the standard phenotype is the corresponding phenotype in a reference cell or population of cells.

38. The method of claim 37, wherein the reference cell is one or more of the following, cells from the subject, cultured cells, cultured cells from the subject, or cells from the subject pre-treatment.

39. The method of claim 38, wherein the cells from the subject are bone marrow stromal cell, (BMSC), a peripheral blood mononuclear cell (PBMC), lymphocytes, hair follicles, blood cells, other epithelial cells, bone marrow plasma cells, primary cancer cells, patient derived tumor cells, normal or cancerous hematopoietic stem cells, neural stem cells, solid tumor cells, or astrocytes.

40. A method of identifying HDAC or TDAC inhibitors, the method comprising steps of:

providing a cell;

providing a test agent;

contacting the cell with a test agent;

treating the cell with a first primary antibody specific for acetylated tubulin;

treating the cell with a second primary antibody specific for acetylated lysine;

treating the cell with a first secondary antibody specific for the first primary antibody and labelled with a unique fluorophore;

~~treating the cell with a second secondary antibody specific for the second primary antibody and labelled with a unique fluorophore, wherein the fluorophore is different from the fluorophore of the first secondary antibody;~~

~~imaging the cells based on the two fluorophores; and~~

~~quantitating the degree of inhibition of both TDAC and HDAC.~~

~~41. The method of claim 40, wherein the cells are derived from a human cancer cell line.~~

~~42. The method of claim 40, wherein the cells are derived from a human multiple myeloma cell line.~~

~~43. A method of identifying a candidate compound to inhibit protein degradation in a cell, comprising:~~

~~contacting a cell exhibiting aggresome formation with a candidate compound, and~~

~~determining a phenotype of the cell, wherein modulation of the phenotype is indicative of the efficacy of the compound.~~

~~44. The method of claim 43, wherein the candidate molecule is one or more of a small molecule, a peptide, or a nucleic acid.~~

~~45. The method of claim 44, wherein the nucleic acid is an RNA, mRNA, RNAi, siRNA or DNA.~~

~~46. The method of claim 43, wherein the peptide is a peptide derived from the HDAC6, the dynein-binding domain of HDAC6, the TDAC domain of HDAC6, the N-terminus of HDAC6 or the C-terminus of HDAC6.~~

~~47. The method of claim 43, wherein the small molecule is contained in a multi-channel arrayer library.~~

~~48. The method of claim 43, wherein the step of determining the phenotype is by image-based multidimensional screening.~~

49. A method for evaluating a test compound, comprising:

contacting an cell exhibiting aggresome formation with a test compound, and

evaluating the cell following contact, wherein a correlation of a modulation of one of more phenotypes to a reference value is an indication that the test compound may be useful as a protein degradation disorder treatment.

50. The method of any one of claims 43 to 49, further comprising determining a phenotype of the cell after an initial period of treatment with the protein degradation inhibitor.

51. The method of any one of claims 43 to 49, wherein the phenotype is a biological or clinical sequelae in response to a particular treatment or compound, including anemia, thrombocytopenia, neutropenia, osteolytic lesions, bone pain, immunodeficiency, renal insufficiency, hypercalcemia, aneuploidy of mature plasma cells, percentage of malignant cells, acetylation state of tubulin, apoptosis of mature plasma cells, level of aggresomes, level of aggresomes in mature plasma cells, HDAC6 ubiquitination, HDAC6 ubiquitination in mature plasma cells, HDAC6 association with dynein in mature plasma cells, cellular levels of ubiquintinated proteins in mature plasma cells, level of caspase-8 in mature plasma cells, level of PARP in mature plasma cells, thymidine uptake in mature plasma cells, dilated ER cisternae, aggregation of mature plasma cells, deposits of immunoglobulins in mature plasma cells, acetylation state of non-histone proteins, global ubiquitination state of the cellular proteins, state of cell cycle regulation, necrosis, markers of apoptosis, apoptosis state, Russell body formation, cystic fibrosis transmembrane protein receptor state, and modulation of cellular protein deposits, or global acetylation state of cellular and extracellular proteins.

52. The method of claim 51, wherein a decrease in one or more of anemia, level of aggresomes, thrombocytopenia, neutropenia, osteolytic lesions, bone pain, immunodeficiency, renal insufficiency, hypercalcemia, aneuploidy of mature plasma cells, percentage of malignant cells, thymidine uptake in mature plasma cells, level of full length caspase-8 in mature plasma cells, level of full length PARP in mature plasma cells, or aggregation of mature plasma cells, indicates that the treatment is efficacious.

53. The method of claim 51, wherein an increase in acetylation state of tubulin, global acetylation state, acetylation state on non-histone proteins, HDAC6 ubiquitination in mature plasma cells, level of cleaved form of caspase-8, level of cleaved form of PARP, necrosis, acetylation state of non-histone proteins, cellular ubiquitination levels, apoptosis, markers of apoptosis, cell cycle deregulation, or deposits of immunoglobulins in mature plasma cells indicates that the treatment is efficacious.

54. The method of claim 51, wherein the evaluating is by image-based multidimensional screening.

55. The method of claim 49, wherein the test compound is one or more of a small molecule, a peptide, or a nucleic acid.

56. The kit, composition or method of any one of claims 1 to 55, wherein the subject is a human or the cell is a human cell.