

CLAIMS

1. An isolated nucleic acid comprising an expression construct encoding an inhibitory nucleic acid that inhibits expression or activity of *ATXN2*, wherein the inhibitory nucleic acid comprises a guide strand sequence comprising the nucleic acid sequence set forth in any one of SEQ ID NOS: 1185, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1184, 1186-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

2. The isolated nucleic acid molecule of claim 1, wherein the inhibitory nucleic acid is a siRNA duplex, shRNA, miRNA, or dsRNA.

3. The isolated nucleic acid molecule of claim 1 or 2, wherein the inhibitory nucleic acid further comprises a passenger strand sequence, optionally wherein the passenger strand sequence is selected from Tables 1, 19, 23, and 24, or a passenger strand sequence selected from Tables 1, 19, 23, and 24 and having 1-10 insertions, deletions, substitutions, mismatches, wobbles, or any combination thereof.

4. The isolated nucleic acid molecule of any one of claims 1-3, wherein the inhibitory nucleic acid is an artificial miRNA, wherein the guide strand sequence and passenger strand sequence are contained within a miRNA backbone sequence.

5. The isolated nucleic acid molecule of claim 4, wherein the miRNA backbone sequence is a miR-100 backbone sequence, a miR-155 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-132 backbone sequence, a miR-9 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence, a miR-16-2 backbone sequence, a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

6. The isolated nucleic acid molecule of any one of claims 1-5, wherein the inhibitory nucleic acid is a miRNA comprising the nucleic acid sequence set forth in any one of SEQ ID NOS: 1915, 443-490, 1109-1111, 1114, 1121-1168, 1405-1520, 1908-1914, 1916-2007, 2011, 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

7. The isolated nucleic acid molecule of any one of claims 1-6, wherein the nucleic acid sequence encoding the inhibitory nucleic acid is located in an untranslated region of the expression construct,

optionally wherein the untranslated region is an intron, a 5' untranslated region (5' UTR), or a 3' untranslated region (3'UTR).

8. The isolated nucleic acid molecule of any one of claims 1-7, further comprising a promoter operably linked to the nucleic acid sequence encoding the inhibitory nucleic acid.

9. The isolated nucleic acid molecule of claim 8, wherein the promoter is a RNA pol III promoter a U6 promoter, a H1 promoter, a chicken-beta actin (CBA) promoter, a CAG promoter, a H1 promoter, a CD68 promoter, a human synapsin promoter, or a JeT promoter,

optionally wherein the H1 promoter comprises nucleotides 113-203 of SEQ ID NO:1522, nucleotides 1798-1888 of SEQ ID NO:1521, nucleotides 244-343 of SEQ ID NO:2257, or nucleotides 113-343 of SEQ ID NO:2257.

10. The isolated nucleic acid molecule of any one of claims 1-9, wherein the expression construct is flanked by a 5' adeno-associated virus (AAV) inverted terminal repeat (ITR) sequence and a 3' AAV ITR sequence, or variants thereof,

optionally wherein one of the ITR sequences lacks a functional terminal resolution site.

11. The isolated nucleic acid molecule of claim 10, wherein:

(a) the 5' and 3' ITRs are derived from an AAV serotype selected from the group consisting of: AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAVRh10, AAV11, and variants thereof; and/or

(b) the 5' ITR comprises nucleotides 1-106 of SEQ ID NO:2257 and the 3' ITR comprises nucleotides 2192-2358 of SEQ ID NO:2257.

12. A vector comprising the isolated nucleic acid molecule of any one of claims 1-11, optionally wherein the vector is a plasmid or viral vector, optionally further wherein the viral vector is a recombinant adeno-associated virus (rAAV) vector or a Baculovirus vector.

13. The vector of claim 12, wherein the rAAV vector is a self-complementary rAAV vector.

14. The vector of claim 12, or 13, wherein the vector further comprises a stuffer sequence.

15. A recombinant adeno-associated (rAAV) particle comprising the isolated nucleic acid molecule of any one of claims 1-11 or the vector of claims 12-14.

16. The rAAV particle of claim 15, wherein the rAAV particle comprises a capsid protein, optionally wherein the capsid protein is capable of crossing the blood-brain barrier.

17. The rAAV particle of claim 15 or 16, wherein:

(a) the capsid protein is an AAV9 capsid protein or AAVrh.10 capsid protein; and/or

(b) the rAAV particle transduces neuronal cells and/or non-neuronal cells of the central nervous system (CNS).

18. A pharmaceutical composition comprising the isolated nucleic acid molecule of any one of claims 1-11, the vector of any one of claims 12-14, or the rAAV particle of any one of claims 15-17, and optionally a pharmaceutically acceptable carrier.

19. A host cell comprising the isolated nucleic acid molecule of any one of claims 1-11, the vector of any one of claims 12-14, or the rAAV particle of any one of claims 15-17.

20. Use of the isolated nucleic acid molecule of any one of claims 1-11, the vector of any one of claims 12-14, the rAAV particle of any one of claims 15-17, or the

pharmaceutical composition of claim 18 in the manufacture of a medicament for treating a subject having or suspected of having a neurodegenerative disease.

21. The use of claim 20, wherein the medicament is to be directly injected to the CNS of the subject, optionally wherein the direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intrastriatal injection, subpial injection, injection to the cerebrospinal fluid (CSF), intracisternal injection, intraventricular injection, or intralumbar injection.

22. The use of claim 20 or 21, wherein the subject is characterized as having an *ATXN2* allele having at least 22 CAG trinucleotide repeats, optionally wherein the *ATXN2* allele has at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats.

23. The use of any one of claims 20-22, wherein the neurodegenerative disease is spinocerebellar ataxia-2, amyotrophic lateral sclerosis, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.

24. An *ex vivo* method of inhibiting *ATXN2* expression in a cell, the method comprising delivering to the cell the isolated nucleic acid of any one of claims 1-11, the vector of any one of claims 12-14, the rAAV particle of any one of claims 15-17, or the pharmaceutical composition of claim 18.

25. The method of claim 24, wherein the cell has an *ATXN2* allele having at least 22 CAG trinucleotide repeats, optionally wherein the *ATXN2* allele has at least 24

CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats.

26. The method of claim 24 or 25, wherein the cell is a cell in the CNS, optionally a neuron, glial cell, astrocyte, or microglial cell.

27. The method of any one of claims 24-26, wherein the cell is from a subject having one or more symptoms of a neurodegenerative disease and/or the cell is from a subject having or suspected of having a neurodegenerative disease, optionally wherein the neurodegenerative disease is spinocerebellar ataxia-2, amyotrophic lateral sclerosis, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.

28. Use of the isolated nucleic acid of any one of claims 1-11, the vector of any one of claims 12-14, the rAAV particle of any one of claims 15-17, or the pharmaceutical composition of claim 18 in the manufacture of a medicament for inhibiting ATXN2 expression in the central nervous system of a subject.

29. The use of claim 28, wherein the medicament is to be directly injected to the CNS of the subject, optionally wherein the direct injection is intracerebral injection, intraparenchymal injection, intrathecal injection, intrastriatal injection, subpial injection, injection to the cerebrospinal fluid (CSF), intracisternal injection, intraventricular injection, or intralumbar injection.

30. The use of claim 28 or 29, wherein the subject has an *ATXN2* allele having at least 24 CAG trinucleotide repeats, at least 27 CAG trinucleotide repeats, at least 30 CAG trinucleotide repeats, or at least 33 or more CAG trinucleotide repeats.

31. An artificial miRNA comprising a guide strand sequence and a passenger strand sequence, wherein the guide strand sequence comprises any one of SEQ ID NOS: 1185, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 1176-1184, 1186-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209.

32. The artificial miRNA of claim 31, wherein the guide strand sequence and passenger strand sequence are contained within a miR backbone sequence.

33. The artificial miRNA of claim 32, wherein the miR backbone sequence is a miR-100 backbone sequence, a miR-155 backbone sequence, a miR-155E backbone sequence, a miR-155M backbone sequence, a miR1-1 backbone sequence, a miR-1-1_M backbone sequence, a miR-16-2 backbone sequence, a miR-100_M backbone sequence, a miR-190a backbone sequence, a miR-190a_M backbone sequence, a miR-124 backbone sequence, a miR-124_M backbone sequence, a miR-132 backbone sequence, a miR-9 backbone sequence, a miR-138-2 backbone sequence, a miR-122 backbone sequence, a miR-122_M backbone sequence, a miR-130a backbone sequence,

or a miR-128 backbone sequence, a miR-144 backbone sequence, a miR-451a backbone sequence, or a miR-223 backbone sequence.

34. The artificial miRNA of any one of claims 31-33, wherein the artificial miRNA comprises the sequence as set forth in any one of SEQ ID NOS: 1915, 443-490, 1109-1111, 1114, 1121-1168, 1405-1520, 1908-1914, 1916-2007, 2011, 2017, 2021, 2025, 2027, 2031, 2035, 2039, 2041, 2045, 2049, 2053, 2057, 2061, 2067, 2071, 2075, 2079, 2085, 2089, 2093, 2097, 2101, 2105, 2109, 2113, 2117, 2120, 2124, 2128, 2132, 2136, 2140, 2144, 2148, 2154, 2158, 2162, 2166, 2170, 2174, 2176, 2180, 2182, 2184, 2187, 2189, 2191, 2193, 2195, 2197, 2199, 2205, 2211, 2261, 2263, 2265, and 2267.

35. An isolated RNA duplex comprising a guide strand sequence and a passenger strand sequence, wherein the guide strand sequence comprises the nucleic acid sequence set forth in any one of SEQ ID NOS: 1185, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, and 1176-1184, 1186-1288, 1811-1827, 2015, 2065, 2083, 2152, 2203, and 2209, optionally wherein the guide strand sequence and passenger strand sequence are linked by a loop region to form a hairpin structure comprising a duplex structure and a loop region, optionally wherein the loop structure comprises from 6 to 25 nucleotides.

36. Use of the artificial miRNA of any one of claims 31-34 or the isolated RNA duplex of claim 35 in the manufacture of a medicament for treating a subject having or suspected of having a neurodegenerative disease.

37. The use of claim 36, wherein the neurodegenerative disease is spinocerebellar ataxia-2, amyotrophic lateral sclerosis, frontotemporal dementia, primary lateral sclerosis, progressive muscular atrophy, limbic-predominant age-related TDP-43 encephalopathy, chronic traumatic encephalopathy, dementia with Lewy bodies, corticobasal degeneration, progressive supranuclear palsy (PSP), dementia Parkinsonism ALS complex of guam (G-PDC), Pick's disease, hippocampal sclerosis, Huntington's disease, Parkinson's disease, or Alzheimer's disease.

38. The isolated nucleic acid of any one of claims 1-11, the vector of any one of claims 12-14, the rAAV particle of any one of claims 15-17, the pharmaceutical composition of claim 18, the artificial miRNA of any one of claims 31-34 or the isolated RNA duplex of claim 35 for use in therapy.