

CLAIMS

What is claimed is:

1. An RNAi comprising a first strand and a second strand, wherein
 - a) the first strand and the second strand form a duplex;
 - b) the first strand comprises a guide region of at least 19 bases, wherein the guide region comprises a seed region comprising bases 1-N of the guide strand, wherein N=7 or N=8; and
 - c) the second strand comprises a non-guide region of at least 19 bases, wherein the non-guide region comprises a bulge sequence opposite base 1 of the guide region in the duplex, and
wherein the bulge is adjacent to the seed region.
2. The RNAi of claim 1, wherein:
 - a) the bulge is formed by one or more bases of the non-guide strand in the duplex that lack a complementary base on the guide region, wherein the bulge is flanked by bases that do basepair with the guide strand;
 - b) the bulge comprises 1 to 10 nucleotides; and/or
 - c) the RNAi comprises a second bulge, wherein the second bulge is located on the first strand in the guide region located 3' to the seed region.
3. The RNAi of claim 1 or 2, wherein the bulge comprises 1-3 nucleotides.
4. An RNAi comprising:
 - (a) a first strand comprising a guide region which comprises the a nucleic acid sequence having about 90% identity to sequence 5'-UAGACAAUGAUUCACACGGU-3' (SEQ ID NO:1) or has the sequence 5'-UAGACAAUGAUUCACACGGU-3' (SEQ ID NO:1) and a second strand comprising a non-guide region which comprises a nucleic acid sequence having about 90% identity to the sequence 5'-ACCGUGUGUCAUUGUCUAA-3' (SEQ ID NO:2) or has sequence 5'-ACCGUGUGUCAUUGUCUAA-3' (SEQ ID NO:2),
wherein the first strand and second strand form a duplex and wherein the A residue at residue 18 or residue 19 of the second strand forms a bulge in the non-guide region;

(b) a first strand comprising a guide region which comprises a nucleic acid sequence having about 90% identity to the sequence 5'-UCGACAAUGAUUCACACGGU-3' (SEQ ID NO:15) or has the sequence 5'-UCGACAAUGAUUCACACGGU-3' (SEQ ID NO:15) and a second strand comprising a non-guide region which comprises a nucleic acid sequence having about 90% identity to the sequence 5'-ACCGUGUGUCAUUGUCGAA-3' (SEQ ID NO:16) or has the sequence 5'-ACCGUGUGUCAUUGUCGAA-3' (SEQ ID NO:16), wherein the first strand and second strand form a duplex and wherein the A residue at residue 18 or residue 19 of the second strand forms a bulge in the non-guide region; or

(c) a first strand comprising a guide region which comprises a nucleic acid sequence having about 90% identity to the sequence 5'-UAGACGAUGAUUCACACGGU-3' (SEQ ID NO:17) or has the sequence 5'-UAGACGAUGAUUCACACGGU-3' (SEQ ID NO:17) and a second strand comprising a non-guide region which comprises a nucleic acid sequence having about 90% identity to the sequence 5'-ACCGUGUGUCAUCGUCUAA-3' (SEQ ID NO:18) or has the sequence 5'-ACCGUGUGUCAUCGUCUAA-3' (SEQ ID NO:18), wherein the first strand and second strand form a duplex and wherein the A residue at residue 18 or residue 19 of the second strand forms a bulge in the non-guide region.

5. The RNAi of claim 4, wherein U residues at residues 11 and 12 of the guide region forms a bulge in the guide region.

6. The RNAi of any one of claims 1-5, wherein:

- (a) the duplex is between 19 and 25 or 19 and 23 base pairs in length;
- (b) the first and/or second strand further comprises a 3' overhang region, a 5' overhang region, or both 3' and 5' overhang regions; and/or
- (c) the first strand and the second strand are linked by means of RNA linker capable of forming a loop structure.

7. The RNAi of claim 6, wherein:

- (a) RNA linker comprises from 4 to 50 nucleotides, 4 to 20 nucleotides, or 13 nucleotides;
- (b) the loop structure comprises 4 to 20 nucleotides; and/or
- (c) the RNAi comprises 5' to 3' the second strand, the RNA linker, and the first strand; or the RNAi comprises 5' to 3' the first strand, the RNA linker, and the second strand.

8. The RNAi of any one of claims 1-7, wherein:
 - (a) the sequence is improved to reduce off-target gene silencing;
 - (b) the sequence comprises one or more CpG motifs; and/or
 - (c) the sequence comprises one or more CpG motifs in the seed region.
9. The RNAi of any one of claims 1-8, wherein the RNAi comprises the nucleotide sequence about 90% identical to the nucleotide sequence of SEQ ID NO:3 or has the nucleotide sequence of SEQ ID NO:3.
10. The RNAi of any one of claims 1-9, wherein the RNAi is a small inhibitory RNA (siRNA), a microRNA (miRNA), or a small hairpin RNA (shRNA).
11. The RNAi of any one of claims 1-10, wherein the RNAi targets RNA encoding a polypeptide associated with a disorder.
12. The RNAi of claim 11, wherein:
 - (a) the disorder is a CNS disorder;
 - (b) the disorder is lysosomal storage disease (LSD), epilepsy, Parkinson's disease, Alzheimer's disease, stroke, corticobasal degeneration (CBD), corticogasal ganglionic degeneration (CBGD), frontotemporal dementia (FTD), multiple system atrophy (MSA), progressive supranuclear palsy (PSP) or cancer of the brain; and/or
 - (c) the disorder is Huntington's disease.
13. The RNAi of claim 12, wherein the polypeptide is huntingtin, wherein:
 - (a) the huntingtin comprises a mutation associated with Huntington's disease; and/or
 - (b) the guide region comprises the sequence 5'-UAGACAAUGAUUCACACGGU-3' (SEQ ID NO:1) and the non-guide region comprises the sequence 5'-ACCGUGUGUCAUUGUCUAA-3' (SEQ ID NO:2); or
 - (c) the guide region comprises the sequence 5'-UCGACAAUGAUUCACACGGU-3' (SEQ ID NO:15) and the non-guide region comprises the sequence 5'-ACCGUGUGUCAUUGUCGAA-3' (SEQ ID NO:16); or
 - (d) the guide region comprises the sequence 5'-UAGACGAUGAUUCACACGGU-3' (SEQ ID NO:17) and the non-guide region comprises the sequence 5'-ACCGUGUGUCAUCGUCUAA-3' (SEQ ID NO:18).

14. A method to reduce the toxicity of a RNAi comprising introducing a bulge in the non-guide region of the RNAi to generate a RNAi of any one of claims 1-13.
15. An expression construct comprising nucleic acid encoding the RNAi of any one of claims 1-13.
16. The expression construct of claim 15 wherein the nucleic acid encoding the RNAi comprises a miRNA scaffold.
17. The expression construct of claim 16, wherein the nucleic acid encoding the RNAi comprises a miR-155 scaffold.
18. The expression construct of any one of claims 15-17, wherein the nucleic acid encoding the RNAi is operably linked to a promoter.
19. The expression construct of claim 18, wherein:
 - (a) the promoter is capable of expressing the RNAi in the brain of a mammal; and/or
 - (b) the promoter is selected from a cytomegalovirus (CMV) immediate early promoter, an RSV LTR, a MoMLV LTR, a phosphoglycerate kinase- 1 (PGK) promoter, a simian virus 40 (SV40) promoter, a CK6 promoter, a transthyretin promoter (TTR), a TK promoter, a tetracycline responsive promoter (TRE), an HBV promoter, an hAAT promoter, a LSP promoter, a chimeric liver-specific promoter (LSP), an E2F promoter, a telomerase (hTERT) promoter; a cytomegalovirus enhancer/chicken beta-actin/Rabbit β -globin promoter (CAG) promoter, an elongation factor 1-alpha promoter (EF1-alpha) promoter, a human β -glucuronidase promoter, a chicken β -actin (CBA) promoter, a retroviral Rous sarcoma virus (RSV) LTR promoter, a dihydrofolate reductase promoter, and a 13-actin promoter.
20. The expression construct of any one of claims 15-19, wherein the expression construct further comprises a polyadenylation signal, wherein optionally the polyadenylation signal is a bovine growth hormone polyadenylation signal, an SV40 polyadenylation signal, or a HSV TK pA.
21. A vector comprising the expression construct of any one of claims 15-20.

22. The vector of claim 21, wherein the vector is a recombinant adeno-associated virus (rAAV) vector, a recombinant adenoviral vector, a recombinant lentiviral vector or a recombinant herpes simplex virus (HSV) vector.

23. The vector of claim 22, wherein

(a) the vector is a recombinant adenoviral vector derived from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3; or

(b) the vector is a recombinant lentiviral vector derived from a lentivirus pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein; or

(c) the vector is a rHSV vector derived from rHSV-1 or rHSV-2; or

24. The vector of claim 22, wherein the vector is a rAAV vector, and wherein the expression construct is flanked by one or more AAV inverted terminal repeat (ITR) sequences.

25. The rAAV vector of claim 24, wherein the expression construct is flanked by two AAV ITRs; and/or

wherein the AAV ITRs are AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV DJ, a goat AAV, bovine AAV, or mouse AAV serotype ITRs.

26. The rAAV vector of claim 24 or 25, wherein the rAAV vector comprise 5' to 3' an AAV2 ITR, a promoter, nucleic acid encoding the RNAi, a polyadenylation signal, and an AAV2 ITR; and wherein:

(a) the promoter is a CBA promoter;

(b) the polyadenylation signal is a bovine growth hormone polyadenylation signal;

and/or

(c) the rAAV vector comprises 5' to 3' an AAV2 ITR, the CBA promoter, nucleic acid encoding the RNAi, a bovine growth hormone polyadenylation signal, and an AAV2 ITR.

27. The rAAV vector of any one of claims 24-26, wherein the vector further comprises a stuffer nucleic acid.

28. The rAAV vector of claim 27, wherein:

(a) the stuffer nucleic acid is located between the promoter and the nucleic acid encoding the RNAi; and/or

(b) the stuffer nucleic acid comprises nucleic acid encoding a green fluorescent protein (GFP), a human alpha-1-antitrypsin (AAT) stuffer sequence, or a C16 P1 chromosome 16 P1 clone (human C16) stuffer sequence, wherein optionally the stuffer nucleic acid comprises a nucleic acid sequence from about nucleotide 1692 to nucleotide 2720 of SEQ ID NO: 13.

29. The rAAV vector of any one of claims 24-28, wherein the vector is a self-complementary rAAV vector.

30. A cell comprising the vector of any one of claims 21-23 or the rAAV vector of any one of claims 24-29.

31. A viral particle comprising the vector of claim 21, wherein the viral particle is an AAV particle encapsidating the rAAV vector, an adenovirus particle encapsidating the recombinant adenoviral vector, a lentiviral particle encapsidating the recombinant lentiviral vector or an HSV particle encapsidating the recombinant HSV vector.

32. The viral particle of claim 31, wherein:

(a) the viral particle is an adenovirus particle encapsidating the recombinant adenoviral vector, wherein the adenovirus particle comprises a capsid from Adenovirus serotype 2, 1, 5, 6, 19, 3, 11, 7, 14, 16, 21, 12, 18, 31, 8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24-30, 37, 40, 41, AdHu2, AdHu 3, AdHu4, AdHu24, AdHu26, AdHu34, AdHu35, AdHu36, AdHu37, AdHu41, AdHu48, AdHu49, AdHu50, AdC6, AdC7, AdC69, bovine Ad type 3, canine Ad type 2, ovine Ad, or porcine Ad type 3; or

(b) the viral particle is a lentiviral particle encapsidating the recombinant lentiviral vector, wherein optionally the lentiviral particle comprises a capsid pseudotyped with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), Ross river virus (RRV), Ebola virus, Marburg virus, Mokala virus, Rabies virus, RD114 or variants therein; or

(c) the viral particle is a HSV particle, wherein the HSV particle is a rHSV-1 particle or a rHSV-2 particle.

33. A recombinant AAV particle comprising the rAAV vector of any one of claims 24-29.

34. The rAAV particle of claim 33, wherein the AAV viral particle comprises an AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVrh8, AAVrh8R, AAV9, AAV10, AAVrh10, AAV11, AAV12, AAV2R471A, AAV2/2-7m8, AAV DJ, AAV2 N587A, AAV2 E548A, AAV2 N708A, AAV V708K, a goat AAV, AAV1/AAV2 chimeric, bovine AAV, or mouse AAV capsid rAAV2/HBoV1 serotype capsid.

35. The rAAV particle of claim 33 or 34, wherein the ITR and the capsid of the rAAV viral particle are derived from the same AAV serotype or are derived from different AAV serotypes.

36. The rAAV particle of any one of claims 33-35, wherein the ITR is derived from AAV2 and the capsid of the rAAV particle is derived from AAV1.

37. A composition comprising the viral particle of claim 31 or 32, or the rAAV particle of any one of claims 33-36, optionally wherein the composition further comprises a pharmaceutically acceptable carrier.

38. A method for inhibiting or reducing the expression of a polypeptide in a mammalian disease comprising administering to the mammal the RNAi of any one of claims 1-13; the expression construct of any one of claims 15-20; the vector of any one of claims 21-23; the rAAV vector of any one of claims 24-29; the viral particle of claim 31 or 32; the rAAV particle of any one of claims 33-36; or the composition of claim 37, wherein the RNAi targets an RNA encoding the polypeptide.

39. A method for inhibiting the accumulation of a polypeptide in a cell of a mammal comprising administering to the mammal the RNAi of any one of claims 1-13; the expression construct of any one of claims 15-20; the vector of any one of claims 21-23; the rAAV vector of any one of claims 24-29; the viral particle of claim 31 or 32; the rAAV particle of any one of claims 33-36; or the composition of claim 37, wherein the RNAi targets an RNA encoding the polypeptide.

40. Use of the RNAi of any one of claims 1-13; the expression construct of any one of claims 15-20; the vector of any one of claims 21-23; the rAAV vector of any one of claims 24-29; the viral particle of claim 31 or 32; the rAAV particle of any one of claims 33-36; or the composition of claim 37, in the manufacture of a medicament for inhibiting or reducing expression of a polypeptide in a mammalian disease, or inhibiting the accumulation of a polypeptide in a cell of a mammal.

41. The RNAi of any one of claims 1-13; the expression construct of any one of claims 15-20; the vector of any one of claims 21-23; the rAAV vector of any one of claims 24-29; the viral particle of claim 31 or 32; the rAAV particle of any one of claims 33-36; or the composition of claim 37 when used in inhibiting or reducing the expression of a polypeptide in a mammalian disease or inhibiting the accumulation of a polypeptide in a cell of a mammal.

42. A kit for inducing RNA interference in a mammal comprising the RNAi of any one of claims 1-13; the expression construct of any one of claims 15-20; the vector of any one of claims 21-23; the rAAV vector of any one of claims 24-29; the viral particle of claims 31 or 32; the rAAV particle of any one of claims 33-36; or the composition of claim 37.