

CLAIMS

WHAT IS CLAIMED IS:

1. A polynucleic acid molecule conjugate comprising:
an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of *DMPK*;
wherein the polynucleic acid molecule having a sense strand having a sequence at least 80% identical to SEQ ID NO: 1, and an antisense strand having a sequence at least 80% identical to SEQ ID NO: 2; and
wherein the polynucleic acid molecule conjugate mediates RNA interference against the *DMPK*.
2. The polynucleic acid molecule conjugate of claim 1, wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) region and a variable light chain (VL) region, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17; HCDR2 sequence EINPIX₁GRSNYAX₂KFQG, wherein X₁ is selected from N or Q and X₂ is selected from Q or E; and HCDR3 sequence comprising SEQ ID NO: 19.
3. The polynucleic acid molecule conjugate of claim 2, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17, HCDR2 sequence comprising one of SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 21, and HCDR3 sequence comprising SEQ ID NO: 19.
4. The polynucleic acid molecule conjugate of any one of claim 2-3, wherein the VL region comprises LCDR1 sequence RTSENIYX₃NLA, LCDR2 sequence AX₄TNLAX₅, and LCDR3 sequence QHFWGTPLTX₆, wherein X₃ is selected from N or S, X₄ is selected from A or G, X₅ is selected from D or E, and X₆ is present or absence, and if present, is F.
5. The polynucleic acid molecule conjugate of any one of claims 2-4, wherein the VL region comprises LCDR1 sequence comprising SEQ ID NO: 22, LCDR2 sequence AATNLAX₅, and LCDR3 sequence QHFWGTPLTX₆, wherein X₅ is selected from D or E and X₆ is present or absence, and if present, is F.
6. The polynucleic acid molecule conjugate of any one of claims 2-5, wherein the VL region comprises LCDR1 sequence comprising SEQ ID NO: 22 or SEQ ID NO: 27, LCDR2

sequence comprising SEQ ID NO: 23, SEQ ID NO: 25, or SEQ ID NO: 28, and LCDR3 sequence comprising SEQ ID NO: 24 or SEQ ID NO: 26.

7. The polynucleic acid molecule conjugate of any one of claims 2-6, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17, HCDR2 sequence comprising SEQ ID NO: 18, and HCDR3 sequence comprising SEQ ID NO: 19; and the VL region comprises LCDR1 sequence comprising SEQ ID NO: 22, LCDR2 sequence comprising SEQ ID NO: 23, and LCDR3 sequence comprising SEQ ID NO: 24.
8. The polynucleic acid molecule conjugate of any one of claims 2-6, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17, HCDR2 sequence comprising SEQ ID NO: 20, and HCDR3 sequence comprising SEQ ID NO: 19; and the VL region comprises LCDR1 sequence comprising SEQ ID NO: 22, LCDR2 sequence comprising SEQ ID NO: 23, and LCDR3 sequence comprising SEQ ID NO: 24.
9. The polynucleic acid molecule conjugate of any one of claims 2-6, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17, HCDR2 sequence comprising SEQ ID NO: 21, and HCDR3 sequence comprising SEQ ID NO: 19; and the VL region comprises LCDR1 sequence comprising SEQ ID NO: 22, LCDR2 sequence comprising SEQ ID NO: 25, and LCDR3 sequence comprising SEQ ID NO: 26.
10. The polynucleic acid molecule conjugate of any one of claims 2-6, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17, HCDR2 sequence comprising SEQ ID NO: 20, and HCDR3 sequence comprising SEQ ID NO: 19; and the VL region comprises LCDR1 sequence comprising SEQ ID NO: 27, LCDR2 sequence comprising SEQ ID NO: 28, and LCDR3 sequence comprising SEQ ID NO: 26.
11. The polynucleic acid molecule conjugate of any one of claims 2-10, wherein the VH region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to a sequence selected from SEQ ID NOs: 29-33.
12. The polynucleic acid molecule conjugate of any one of claims 2-11, wherein the VL region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to a sequence selected from SEQ ID NOs: 34-38.
13. The polynucleic acid molecule conjugate of any one of claims 2-12, wherein the VH region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO:

30, and wherein the VL region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO: 34.

14. The polynucleic acid molecule conjugate of any one of claims 1-13, wherein the anti-transferrin receptor antibody comprises a humanized antibody, or antigen binding fragment thereof or a chimeric antibody or antigen binding fragment thereof, or a multi-specific antibody or antigen binding fragment thereof.
15. The polynucleic acid molecule conjugate of any one of claims 1-14, wherein the anti-transferrin receptor antibody comprises an IgG-scFv, nanobody, BiTE, diabody, DART, TandAb, scDiabody, scDiabody-CH3, triple body, mini-antibody, minibody, TriBi minibody, scFv-CH3 KIH, Fab-scFv-Fc KIH, Fab-scFv, scFv-CH-CL-scFv, F(ab')₂, F(ab')₂-scFv2, scFv-KIH, Fab-scFv-Fc, tetravalent HCAb, scDiabody-Fc, diabody-Fc, tandem scFv-Fc, or intrabody.
16. The polynucleic acid molecule conjugate of any one of claims 1-15, wherein the anti-transferrin receptor antibody comprises an IgG1 framework.
17. The polynucleic acid molecule conjugate of any one of claims 1-15, wherein the anti-transferrin receptor antibody comprises an IgG2 framework.
18. The polynucleic acid molecule conjugate of claim 17, wherein the IgG2 framework is IgG2b framework.
19. The polynucleic acid molecule conjugate of any one of claims 1-15, wherein the anti-transferrin receptor antibody comprises IgG4 framework.
20. The polynucleic acid molecule conjugate of any one of claims 1-19, wherein the anti-transferrin receptor antibody further comprises at least one mutation in the Fc region.
21. The polynucleic acid molecule conjugate of any one of claims 1-20, wherein the at least one mutation modulates effector function.
22. The polynucleic acid molecule conjugate of any one of claims 20-21, wherein the at least one mutation attenuates or eliminates Fc- γ receptor binding.
23. The polynucleic acid molecule conjugate of any one of claims 20-22, wherein the at least one mutation is at residue position D265, N297, K322, L328, or P329, wherein the residue position is in reference to IgG1.

24. The polynucleic acid molecule conjugate of any one of claims 20-23, wherein the Fc region comprises two or more, three or more, or four or more mutations.
25. The polynucleic acid molecule conjugate of any one of claims 20-24, wherein the Fc region comprises mutations at L233 and L234, wherein the residues correspond to position 233 and 234 of SEQ ID NO: 39.
26. The polynucleic acid molecule conjugate of any one of claims 20-25, wherein the Fc region comprises mutations at D265 and N297.
27. The polynucleic acid molecule conjugate of any one of claims 1-26, wherein the anti-transferrin receptor antibody comprises a heavy chain (HC) sequence selected from SEQ ID NOs: 39-62 and a light chain (LC) sequence selected from SEQ ID NOs: 63-66.
28. The polynucleic acid molecule conjugate of any one of claims 1-27, wherein the anti-transferrin receptor antibody specifically binds to human transferrin receptor (TfR).
29. The polynucleic acid molecule conjugate of any one of claims 1-28, wherein the sense strand and the antisense strand each independently comprises at least one 2' modified nucleotide, at least one modified internucleotide linkage, or at least one inverted abasic moiety.
30. The polynucleic acid molecule conjugate of any one of claims 1-29, wherein the sense strand comprises a 2'-O-methyl modified nucleotide at the 5'-end.
31. The polynucleic acid molecule conjugate of any one of claims 1-30, wherein the sense strand comprises at least two consecutive 2'-O-methyl modified nucleotides at the 5'-end.
32. The polynucleic acid molecule conjugate of any one of claims 1-31, wherein the sense strand comprises at least three, four, five, or six consecutive 2'-O-methyl modified nucleotides at the 5'-end.
33. The polynucleic acid molecule conjugate of any one of claims 1-32, wherein the sense strand comprises six consecutive 2'-O-methyl modified nucleotides at the 5'-end.
34. The polynucleic acid molecule conjugate of any one of claims 1-33, wherein the sense strand comprises at least one 2'-F modified nucleotides.
35. The polynucleic acid molecule conjugate of any one of claims 1-33, wherein the sense strand comprises at least two, at least three 2'-F modified nucleotides.

36. The polynucleic acid molecule conjugate of any one of claims 1-33, wherein the sense strand comprises at least two, at least three consecutive 2'-F modified nucleotides.
37. The polynucleic acid molecule conjugate of any one of claims 1-36, wherein the sense strand comprises a 2'-O-methyl modified nucleotide at the 3'-end.
38. The polynucleic acid molecule conjugate of any one of claims 1-37, wherein the sense strand comprises at least two consecutive 2'-O-methyl modified nucleotides at the 3'-end.
39. The polynucleic acid molecule conjugate of any one of claims 1-38, wherein the sense strand comprises at least three, four, five, six, seven, eight, nine, or ten consecutive 2'-O-methyl modified nucleotides at the 3'-end.
40. The polynucleic acid molecule conjugate of any one of claims 1-39, wherein the sense strand comprises ten consecutive 2'-O-methyl modified nucleotides at the 3'-end.
41. The polynucleic acid molecule conjugate of any one of claims 1-40, wherein the sense strand comprises at least two phosphorothioate internucleotide linkages.
42. The polynucleic acid molecule conjugate of any one of claims 1-41, wherein the sense strand has a sequence of SEQ ID NO: 3, 5, 7, 9, 11, 13 or 15.
43. The polynucleic acid molecule conjugate of any one of claims 1-42, wherein the antisense strand comprises a 2'-O-methyl modified nucleotide at the 5'-end.
44. The polynucleic acid molecule conjugate of any one of claims 1-43, wherein the antisense strand comprises a 2'-O-methyl modified nucleotide at the 3'-end.
45. The polynucleic acid molecule conjugate of any one of claims 1-44, wherein the antisense strand comprises at least two, at least three, at least four, at least five consecutive 2'-O-methyl modified nucleotide at the 3'-end.
46. The polynucleic acid molecule conjugate of any one of claims 1-45, wherein the antisense strand comprises five consecutive 2'-O-methyl modified nucleotide at the 3'-end.
47. The polynucleic acid molecule conjugate of any one of claims 1-46, wherein the antisense strand comprises at least one, at least two, at least three, at least four 2'-F modified nucleotides.

48. The polynucleic acid molecule conjugate of any one of claims 1-47, wherein the antisense strand comprises four 2'-F modified nucleotides, wherein any two of the four 2'-F modified nucleotides are not consecutive.
49. The polynucleic acid molecule conjugate of any one of claims 1-48, wherein the antisense strand comprises two overhang nucleotides at the 3'-end.
50. The polynucleic acid molecule conjugate of any one of claims 1-49, wherein the antisense strand comprises at least two, at least three phosphorothioate internucleotide linkages.
51. The polynucleic acid molecule conjugate of any one of claims 1-50, wherein the antisense strand has a sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14, or 16.
52. The polynucleic acid molecule conjugate of any one of claims 1-51, wherein the polynucleic acid molecule conjugate comprises a linker connecting anti-transferrin receptor antibody or antigen binding fragment thereof to the polynucleic acid molecule.
53. The polynucleic acid molecule conjugate of claim 52, wherein the linker is C6 linker.
54. The polynucleic acid molecule conjugate of claim 53, wherein the C6 linker is a 6-Amino-1-hexanol linker.
55. The polynucleic acid molecule conjugate of claim 52, wherein the linker is a homobifunctional linker or heterobifunctional linker, a maleimide group, a dipeptide moiety, a benzoic acid group, or its derivative thereof.
56. The polynucleic acid molecule conjugate of any one of claims 52-55, wherein the linker comprises 4-(N-maleimidomethyl) cyclohexane -1-amidate (SMCC).
57. The polynucleic acid molecule conjugate of any one of claims 52-56, wherein the linker is coupled to the 5'-end of the sense strand.
58. The polynucleic acid molecule conjugate of any one of claims 52-57, wherein the polynucleic acid molecule is conjugated to a cysteine residue of the anti-transferrin receptor antibody or antigen binding fragment thereof.
59. The polynucleic acid molecule conjugate of claim 58, wherein the cysteine residue is in the Fc domain of the anti-transferrin receptor antibody or antigen binding fragment thereof.

60. The polynucleic acid molecule conjugate of any one of claims 1-59, wherein a ratio between the polynucleic acid molecule and the anti-transferrin receptor antibody or antigen binding fragment thereof is about 1:1, 2:1, 3:1, or 4:1.
61. The polynucleic acid molecule conjugate of any one of claims 1-60, wherein the polynucleic acid moiety mediates RNA interference against the human DMPK gene modulates muscle atrophy in a subject.
62. The polynucleic acid molecule conjugate of claim 61, wherein the RNA interference comprises reducing expression of the mRNA transcript of DMPK gene at least 50%, at least 60%, or at least 70% compared to a quantity of the mRNA transcript of DMPK gene in a cell affected by a muscle dystrophy.
63. The polynucleic acid molecule conjugate of any one of claims 61-62, wherein the muscle dystrophy is myotonic dystrophy type 1 (DM1).
64. A polynucleic acid molecule conjugate comprising:
an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of *DMPK*;
wherein the polynucleic acid molecule having a sense strand having a sequence of SEQ ID NO: 3, 5, 7, 9, 11, 13, or 15 and an antisense strand having a sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14, or 16;
wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) region and a variable light chain (VL) region, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17, HCDR2 sequence comprising SEQ ID NO: 20, and HCDR3 sequence comprising SEQ ID NO: 19; and the VL region comprises LCDR1 sequence comprising SEQ ID NO: 22, LCDR2 sequence comprising SEQ ID NO: 23, and LCDR3 sequence comprising SEQ ID NO: 24; and
wherein the anti-transferrin receptor antibody or antigen binding fragment thereof and the polynucleic acid molecule is conjugated via a linker comprising 4-(N-maleimidomethyl) cyclohexane -1-amidate (SMCC).
65. A polynucleic acid molecule conjugate comprising:
an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of *DMPK*;

wherein the polynucleic acid molecule having a sense strand having a sequence of SEQ ID NO: 3, 5, 7, 9, 11, 13, or 15 and an antisense strand having a sequence of SEQ ID NO: 4, 6, 8, 10, 12, 14, or 16;

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) region and a variable light chain (VL) region, wherein the VH region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO: 30, and wherein the VL region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO: 34; and

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof and the polynucleic acid molecule is conjugated via a maleimide linker.

66. A polynucleic acid molecule conjugate comprising:

an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of *DMPK*;

wherein the polynucleic acid molecule having a sense strand having a sequence of SEQ ID NO: 1 and an antisense strand having a sequence of SEQ ID NO: 2;

wherein the sense strand comprises at least three, four, five, or six consecutive 2'-O-methyl modified nucleotides at the 5'-end and at least two, at least three 2'-F modified nucleotides;

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) region and a variable light chain (VL) region, wherein the VH region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO: 30, and wherein the VL region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO: 34; and

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof and the polynucleic acid molecule is conjugated via a maleimide linker.

67. A polynucleic acid molecule conjugate comprising:

an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of *DMPK*;

wherein the polynucleic acid molecule having a sense strand having a sequence of SEQ ID NO: 1 and an antisense strand having a sequence of SEQ ID NO: 2;

wherein the antisense strand comprises at least two, at least three, at least four, at least five consecutive 2'-O-methyl modified nucleotide at the 3'-end, and at least one, at least two, at least three, at least four 2'-F modified nucleotides;

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) region and a variable light chain (VL) region, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17, HCDR2 sequence comprising SEQ ID NO: 20, and HCDR3 sequence comprising SEQ ID NO: 19; and the VL region comprises LCDR1 sequence comprising SEQ ID NO: 22, LCDR2 sequence comprising SEQ ID NO: 23, and LCDR3 sequence comprising SEQ ID NO: 24; and

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof and the polynucleic acid molecule is conjugated via a maleimide linker.

68. A polynucleic acid molecule conjugate comprising:

an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of *DMPK*;

wherein the polynucleic acid molecule having a sense strand having a sequence of SEQ ID NO: 1 and an antisense strand having a sequence of SEQ ID NO: 2;

wherein the antisense strand comprises 2'-O-methyl modified nucleotides at the 5'-end and at the 3'-end;

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) region and a variable light chain (VL) region, wherein the VH region comprises HCDR1 sequence comprising SEQ ID NO: 17, HCDR2 sequence comprising SEQ ID NO: 18, and HCDR3 sequence comprising SEQ ID NO: 19; and the VL region comprises LCDR1 sequence comprising SEQ ID NO: 22, LCDR2 sequence comprising SEQ ID NO: 23, and LCDR3 sequence comprising SEQ ID NO: 24; and

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof and the polynucleic acid molecule is conjugated via a maleimide linker.

69. A polynucleic acid molecule conjugate comprising:

an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a polynucleic acid molecule that hybridizes to a target sequence of *DMPK*;

wherein the polynucleic acid molecule having a sense strand having a sequence of SEQ ID NO: 1 and an antisense strand having a sequence of SEQ ID NO: 2;

wherein the antisense strand comprises at least five consecutive 2'-O-methyl modified nucleotide at the 3'-end and four 2'-F modified nucleotides, wherein any two of the four 2'-F modified nucleotides are not consecutive;

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) region and a variable light chain (VL) region, and the VH region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO: 30, and wherein the VL region comprises at least 80%, 85%, 90%, 95%, 99%, or 100% sequence identity to SEQ ID NO: 34; and

wherein the anti-transferrin receptor antibody or antigen binding fragment thereof and the polynucleic acid molecule is conjugated via a 6-Amino-1-hexanol linker.

70. A pharmaceutical composition comprising:

a polynucleic acid molecule conjugate of any one of claims 1-69; and
a pharmaceutically acceptable excipient.

71. The pharmaceutical composition of claim 70, wherein the pharmaceutical composition is formulated as a nanoparticle formulation.

72. The pharmaceutical composition of any one of claims 70-71, wherein the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, or transdermal administration.

73. A method for treating muscular dystrophy in a subject in need thereof, comprising:

providing a polynucleic acid conjugate of any one of claims 1-69 or a pharmaceutical composition of any one of claims 70-72; and
administering the polynucleic acid conjugate to the subject in need thereof to treat the muscular dystrophy, wherein the polynucleic acid conjugate reduces a quantity of the mRNA transcript of human *DMPK*.

74. The method of claim 73, wherein the polynucleic acid moiety mediates RNA interference against the human *DMPK* modulates muscle atrophy in a subject.

75. The method of any one of claims 73-74, wherein the muscular dystrophy is myotonic dystrophy type 1 (DM1).

76. Use of the polynucleic acid molecule conjugate of any one of claims 1-69 or the pharmaceutical composition of any one of claims 70-72 for treating in a subject diagnosed with or suspected to have myotonic dystrophy type 1 (DM1).
77. Use of the polynucleic acid molecule conjugate of any one of claims 1-69 or the pharmaceutical composition of any one of claims 70-72 for manufacturing a medicament for treating in a subject diagnosed with or suspected to have myotonic dystrophy type 1 (DM1).
78. A kit comprising a polynucleic acid molecule conjugate of claims 1-69 or the pharmaceutical composition of any one of claims 70-72.