

COMPOSITIONS AND METHODS OF TREATING MUSCLE DYSTROPHY

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 63/001,211 filed March 27, 2020, which is incorporated herein by reference in its entirety.

BACKGROUND OF THE DISCLOSURE

[0002] Gene suppression by RNA-induced gene silencing provides several levels of control: transcription inactivation, small interfering RNA (siRNA)-induced mRNA degradation, and siRNA-induced transcriptional attenuation. In some instances, RNA interference (RNAi) provides long lasting effect over multiple cell divisions. As such, RNAi represents a viable method useful for drug target validation, gene function analysis, pathway analysis, and disease therapeutics.

INCORPORATION BY REFERENCE

[0003] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

SUMMARY

[0003a] Disclosed herein, in one aspect, is a small interfering RNA (siRNA) molecule conjugate comprising an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a siRNA molecule that hybridizes to a target sequence of Dystrophia Myotonica Protein Kinase (*DMPK*) mRNA; wherein the siRNA molecule comprises a sense strand comprising SEQ ID NO: 3 and an antisense strand comprising SEQ ID NO: 4; and wherein the siRNA molecule conjugate mediates RNA interference against *DMPK*.

[0003b] Disclosed herein, in another aspect, is a pharmaceutical composition comprising: a. the siRNA molecule conjugate disclosed herein; and b. a pharmaceutically acceptable excipient.

[0003c] Disclosed herein, in another aspect, is a use of the siRNA molecule conjugate disclosed herein or the pharmaceutical composition disclosed herein in the manufacture of a medicament for treating muscular dystrophy in a subject, wherein the subject is to be administered a therapeutically effective amount of the siRNA molecule conjugate or the pharmaceutical composition.

[0003d] Disclosed herein, in another aspect, is the siRNA molecule conjugate disclosed herein or the pharmaceutical composition disclosed herein for use in therapy.

[0004] Disclosed herein, in certain embodiments, are polynucleic acid molecules and pharmaceutical compositions for modulating a gene associated with muscle dystrophy (*e.g.*,

DM1). In some embodiments, also described herein are methods of treating muscle atrophy with a polynucleic acid molecule or a polynucleic acid molecule conjugate disclosed herein.

[0005] Disclosed herein, in certain embodiments, is 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*. 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. The polynucleic acid molecule conjugate mediates RNA interference against the *DMPK*.

[0006] In some embodiments, 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. In some embodiments, 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

CLAIMS

WHAT IS CLAIMED IS:

1. A small interfering RNA (siRNA) molecule conjugate comprising an anti-transferrin receptor antibody or antigen binding fragment thereof conjugated to a siRNA molecule that hybridizes to a target sequence of Dystrophia Myotonica Protein Kinase (DMPK) mRNA; wherein the siRNA molecule comprises a sense strand comprising SEQ ID NO: 3 and an antisense strand comprising SEQ ID NO: 4; and wherein the siRNA molecule conjugate mediates RNA interference against DMPK.
2. The siRNA molecule conjugate of claim 1, wherein the anti-transferrin receptor antibody or antigen binding fragment thereof is a humanized antibody or antigen binding fragment thereof, a chimeric antibody or antigen binding fragment thereof, or a multi-specific antibody or antigen binding fragment thereof.
3. The siRNA molecule conjugate of claim 2, wherein the anti-transferrin receptor antibody or antigen binding fragment thereof 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.
4. The siRNA molecule conjugate of any one of claims 1-3, wherein the anti-transferrin receptor antibody or antigen binding fragment thereof specifically binds to human transferrin receptor (TfR).
5. The siRNA molecule conjugate of any one of claims 1-4, wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) region, which comprises an HCDR1 comprising SEQ ID NO: 17; an HCDR2 comprising SEQ ID NO: 20; and an HCDR3 comprising SEQ ID NO: 19; and wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a

variable light chain (VL) region, which comprises an LCDR1 comprising SEQ ID NO: 22; an LCDR2 comprising SEQ ID NO: 23; and an LCDR3 comprising SEQ ID NO: 24.

6. The siRNA molecule conjugate of any one of claims 1-5, wherein the anti-transferrin receptor antibody or antigen binding fragment thereof comprises a variable heavy chain (VH) sequence of SEQ ID NO: 30 and a variable light chain (VL) sequence of SEQ ID NO: 34.
7. The siRNA molecule conjugate of any one of claims 1-6, wherein the anti-transferrin receptor antibody comprises a heavy chain sequence of SEQ ID NO: 48 and a light chain sequence of SEQ ID NO: 63.
8. The siRNA molecule conjugate of any one of claims 1-7, wherein the anti-transferrin receptor antibody comprises two heavy chains, each of which comprises SEQ ID NO:48, and two light chains, each of which comprises SEQ ID NO:63.
9. The siRNA molecule conjugate of any one of claims 1-8, wherein the anti-transferrin receptor antibody or antigen binding fragment thereof is conjugated to the siRNA molecule via a linker.
10. The siRNA molecule conjugate of claim 9, wherein the linker comprises a homobifunctional linker or a heterobifunctional linker.
11. The siRNA molecule conjugate of claim 9 or claim 10, wherein the linker is a cleavable linker or a non-cleavable linker.
12. The siRNA molecule conjugate of any one of claims 9-11, wherein the linker is conjugated to the sense strand of the siRNA molecule.
13. The siRNA molecule conjugate of claim 12, wherein the linker is conjugated to the 5' end of the sense strand of the siRNA molecule.

14. The siRNA molecule conjugate of any one of claims 1-13, wherein the siRNA molecule is conjugated to a cysteine residue of the anti-transferrin receptor antibody or antigen binding fragment thereof.
15. The siRNA molecule conjugate of any one of claims 1-14, wherein a ratio between the siRNA molecule and the anti-transferrin receptor antibody or antigen binding fragment thereof is about 1:1, 2:1, 3:1, or 4:1.
16. A pharmaceutical composition comprising:
 - a. the siRNA molecule conjugate of any one of claims 1-15; and
 - b. a pharmaceutically acceptable excipient.
17. The pharmaceutical composition of claim 16, wherein the pharmaceutical composition is formulated for parenteral, oral, intranasal, buccal, rectal, intrathecal, or transdermal administration.
18. The pharmaceutical composition of claim 16, wherein the pharmaceutical composition is formulated for intravenous administration.
19. Use of the siRNA molecule conjugate of any one of claims 1-15 or the pharmaceutical composition of any one of claims 16-18 in the manufacture of a medicament for treating muscular dystrophy in a subject, wherein the subject is to be administered a therapeutically effective amount of the siRNA molecule conjugate or the pharmaceutical composition.
20. The use of claim 19, wherein the medicament is to be administered parenterally, orally, intranasally, buccally, rectally, intrathecally, intravenously, or transdermally.
21. The use of claim 19 or 20, wherein the medicament is to be administered intravenously.

22. The use of any one of claims 19-21, wherein the muscular dystrophy is myotonic dystrophy type 1 (DM1).
23. The siRNA molecule conjugate of any one of claims 1-15 or the pharmaceutical composition of any one of claims 16-18 for use in therapy.