

CLAIMS

What is claimed is:

1. A complex comprising an anti-transferrin receptor antibody covalently linked to an exon-skipping oligonucleotide,

wherein the anti-transferrin receptor antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region comprise human or humanized framework regions; wherein the anti-transferrin receptor antibody binds in the range of C89 to F760 of human transferrin receptor protein 1 (TfR1) having an amino acid sequence as set forth in SEQ ID NO: 1;

wherein the oligonucleotide of the complex is 30 nucleotides in length and comprises a region of complementarity of 25, 26, 27, 28, 29, or 30 nucleotides in length, wherein the region of complementarity is complementary to the annealing site of an oligonucleotide consisting of the sequence of SEQ ID NO: 195, wherein the oligonucleotide of the complex is a phosphorodiamidate morpholino oligomer (PMO); and

wherein the oligonucleotide of the complex is covalently linked at the 5' end to a lysine residue of the anti-transferrin receptor antibody via a cleavable linker comprising a valine-citrulline sequence.

2. The complex of claim 1, wherein the anti-transferrin receptor antibody is in the form of a Fab fragment.

3. The complex of claim 1 or claim 2, wherein the region of complementarity is 30 nucleotides in length.

4. The complex of any one of claims 1 to 3, wherein the oligonucleotide of the complex is 30 nucleotides in length, and fully complementary to the annealing site of the oligonucleotide consisting of the sequence of SEQ ID NO: 195.

5. The complex of any one of claims 1 to 4, wherein the cleavable linker comprises a triazole covalently linked to the valine-citrulline sequence and further covalently linked to a lysine residue of the anti-transferrin receptor antibody,

wherein the triazole is obtained by a cycloaddition reaction between an azide and an alkyne to form the triazole, and

wherein prior to the cycloaddition reaction, the azide is covalently linked to the valine-citrulline sequence of the cleavable linker that is covalently linked to the 5' end of the oligonucleotide of the complex, and the alkyne is provided in a bicyclononyne moiety.

6. The complex of any one of claims 1 to 5, wherein the anti-transferrin receptor antibody is not glycosylated.

7. The complex of any one of claims 1 to 6, wherein the nucleobases of the region of complementarity are selected from adenosine, thymidine, guanosine, and cytosine.

8. A composition comprising the complex of any one of claims 1 to 7 and a pharmaceutically acceptable carrier, wherein the composition is formulated for intravenous administration.

9. A complex according to any one of claims 1-7 for use as a medicament for inducing skipping of exon 51 of a DMD pre-mRNA in a muscle cell of a subject, wherein the complex is to be administered to the subject.

10. A complex for use as a medicament for treating Duchenne muscular dystrophy in a subject in need thereof, wherein a complex comprising an anti-transferrin receptor antibody covalently linked to an exon-skipping oligonucleotide is to be administered to the subject,

wherein the anti-transferrin receptor antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region comprise human or humanized framework regions; wherein the anti-transferrin receptor antibody binds in the range of C89 to F760 of human transferrin receptor protein 1 (TfR1) having an amino acid sequence as set forth in SEQ ID NO: 1;

wherein the oligonucleotide of the complex is 30 nucleotides in length and comprises a region of complementarity of 25, 26, 27, 28, 29, or 30 nucleotides in length, wherein the region of complementarity is complementary to the annealing site of an oligonucleotide consisting of

the sequence of SEQ ID NO: 195, wherein the oligonucleotide is a phosphorodiamidate morpholino oligomer (PMO); and

wherein the oligonucleotide of the complex is covalently linked at the 5' end to a lysine residue of the anti-transferrin receptor antibody via a cleavable linker comprising a valine-citrulline sequence.

11. The complex for use according to claim 10, wherein the region of complementarity is 30 nucleotides in length and fully complementary to the annealing site of the oligonucleotide consisting of the sequence of SEQ ID NO: 195.

12. The complex for use according to claim 10 or claim 11, and wherein the anti-transferrin receptor antibody is in the form of a Fab fragment.

13. The complex for use according to any one of claims 10 to 12, wherein the cleavable linker comprises a triazole covalently linked to the valine-citrulline sequence and further covalently linked to a lysine residue of the anti-transferrin receptor antibody,

wherein the triazole is obtained by a cycloaddition reaction between an azide and an alkyne to form the triazole, and

wherein prior to the cycloaddition reaction, the azide is covalently linked to the valine-citrulline sequence of the cleavable linker that is covalently linked to the 5' end of the oligonucleotide of the complex, and the alkyne is provided in a bicyclononyne moiety.

14. The complex for use according to any one of claims 10 to 13, wherein the oligonucleotide of the complex promotes the expression or activity of a functional dystrophin protein in a muscle cell of the subject.

15. The complex for use according to claim 14, wherein the muscle cell is a skeletal muscle cell or a cardiac muscle cell.

16. The complex for use according to any one of claims 10 to 15, wherein the nucleobases of the region of complementarity are selected from adenosine, thymidine, guanosine, and cytosine.

17. The complex for use according to any one of claims 10 to 16, wherein the subject is human.

18. The complex for use according to any one of claims 10 to 17, wherein the complex is to be administered to the subject intravenously.

19. A complex comprising an anti-transferrin receptor antibody covalently linked to an exon-skipping oligonucleotide,

wherein the anti-transferrin receptor antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region comprise human or humanized framework regions; wherein the anti-transferrin receptor antibody binds in the range of C89 to F760 of human transferrin receptor protein 1 (TfR1) having an amino acid sequence as set forth in SEQ ID NO: 1;

wherein the oligonucleotide is 30 nucleotides in length, wherein the oligonucleotide is a phosphorodiamidate morpholino oligomer (PMO) comprising a region of complementarity fully complementary to the annealing site of an oligonucleotide consisting of the sequence of SEQ ID NO: 195, wherein the nucleobases of the region of complementarity are selected from adenosine, thymidine, guanosine, and cytosine; and

wherein the oligonucleotide of the complex is covalently linked at the 5' end to a lysine residue of the anti-transferrin receptor antibody via a cleavable linker comprising a valine-citrulline sequence.

20. The complex of claim 19, wherein the anti-transferrin receptor antibody is in the form of a Fab fragment.

21. The complex of claim 19 or claim 20, wherein the cleavable linker comprises a triazole covalently linked to the valine-citrulline sequence and further covalently linked to a lysine residue of the anti-transferrin receptor antibody,

wherein the triazole is obtained by a cycloaddition reaction between an azide and an alkyne to form the triazole, and

wherein prior to the cycloaddition reaction, the azide is covalently linked to the valine-citrulline sequence of the cleavable linker that is covalently linked to the 5' end of the oligonucleotide and the alkyne is provided in a bicyclononyne moiety.

22. The complex of any one of claims 19 to 21, wherein the anti-transferrin receptor antibody is not glycosylated.

23. A composition comprising the complex of any one of claims 19 to 21 and a pharmaceutically acceptable carrier, wherein the composition is formulated for intravenous administration.

24. A complex for the use as a medicament for inducing skipping of exon 51 of a DMD pre-mRNA in a muscle cell of a subject, wherein the complex of any one of claims 19 to 21 is to be administered to the subject.

25. A complex for the use as a medicament for treating Duchenne muscular dystrophy in a subject in need thereof, wherein a complex comprising an anti-transferrin receptor antibody covalently linked to an exon-skipping oligonucleotide is to be administered to the subject,

wherein the anti-transferrin receptor antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region comprise human or humanized framework regions; wherein the anti-transferrin receptor antibody binds in the range of C89 to F760 of human transferrin receptor protein 1 (TfR1) having an amino acid sequence as set forth in SEQ ID NO: 1;

wherein the oligonucleotide is 30 nucleotides in length, wherein the oligonucleotide is a phosphorodiamidate morpholino oligomer (PMO) comprising a region of complementarity fully complementary to the annealing site of an oligonucleotide consisting of the sequence of SEQ ID NO: 195, wherein the nucleobases of the region of complementarity are selected from adenosine, thymidine, guanosine, and cytosine; and

wherein the oligonucleotide is covalently linked at the 5' end to a lysine residue of the anti-transferrin receptor antibody via a cleavable linker comprising a valine-citrulline sequence.

26. The complex for use according to claim 25, wherein the cleavable linker comprises a triazole covalently linked to the valine-citrulline sequence and further covalently linked to a lysine residue of the anti-transferrin receptor antibody,
wherein the triazole is obtained by a cycloaddition reaction between an azide and an alkyne to form the triazole, and
wherein prior to the cycloaddition reaction, the azide is covalently linked to the valine-citrulline sequence of the cleavable linker that is covalently linked to the 5' end of the oligonucleotide and the alkyne is provided in a bicyclononyne moiety.
27. The complex for use according to claim 25 or claim 26, wherein the anti-transferrin receptor antibody is not glycosylated.
28. The complex for use according to any one of claims 25 to 27, wherein the oligonucleotide of the complex promotes the expression or activity of a functional dystrophin protein in a muscle cell of the subject.
29. The complex for use according to claim 28, wherein the muscle cell is a skeletal muscle cell or a cardiac muscle cell.
30. The complex for use according to any one of claims 25 to 29, wherein the subject is human.
31. The complex for use according to any one of claims 25 to 30, wherein the complex is to be administered to the subject intravenously.
32. Use of a complex according to any one of claims 1-7 in the manufacture of a medicament for inducing skipping of exon 51 of a DMD pre-mRNA in a muscle cell of a subject.
33. Use of a complex in the manufacture of a medicament for treating Duchenne muscular dystrophy in a subject in need thereof, wherein a complex comprising an anti-transferrin receptor

antibody covalently linked to an exon-skipping oligonucleotide is to be administered to the subject,

wherein the anti-transferrin receptor antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region comprise human or humanized framework regions; wherein the anti-transferrin receptor antibody binds in the range of C89 to F760 of human transferrin receptor protein 1 (TfR1) having an amino acid sequence as set forth in SEQ ID NO: 1;

wherein the oligonucleotide of the complex is 30 nucleotides in length and comprises a region of complementarity of 25, 26, 27, 28, 29, or 30 nucleotides in length, wherein the region of complementarity is complementary to the annealing site of an oligonucleotide consisting of the sequence of SEQ ID NO: 195, wherein the oligonucleotide is a phosphorodiamidate morpholino oligomer (PMO); and

wherein the oligonucleotide of the complex is covalently linked at the 5' end to a lysine residue of the anti-transferrin receptor antibody via a cleavable linker comprising a valine-citrulline sequence.

34. The use according to claim 33, wherein the region of complementarity is 30 nucleotides in length and fully complementary to the annealing site of the oligonucleotide consisting of the sequence of SEQ ID NO: 195.

35. The use according to claim 33 or claim 34, and wherein the anti-transferrin receptor antibody is in the form of a Fab fragment.

36. The use according to any one of claims 33 to 35, wherein the cleavable linker comprises a triazole covalently linked to the valine-citrulline sequence and further covalently linked to a lysine residue of the anti-transferrin receptor antibody,

wherein the triazole is obtained by a cycloaddition reaction between an azide and an alkyne to form the triazole, and

wherein prior to the cycloaddition reaction, the azide is covalently linked to the valine-citrulline sequence of the cleavable linker that is covalently linked to the 5' end of the oligonucleotide of the complex, and the alkyne is provided in a bicyclononyne moiety.

37. The use according to any one of claims 33 to 36, wherein the oligonucleotide of the complex promotes the expression or activity of a functional dystrophin protein in a muscle cell of the subject.
38. The use according to claim 37, wherein the muscle cell is a skeletal muscle cell or a cardiac muscle cell.
39. The use according to any one of claims 33 to 38, wherein the nucleobases of the region of complementarity are selected from adenosine, thymidine, guanosine, and cytosine.
40. The use according to any one of claims 33 to 39, wherein the subject is human.
41. The use according to any one of claims 33 to 40, wherein the complex is to be administered to the subject intravenously.
42. Use of a complex according to any one of claims 19 to 21 in the manufacture of a medicament for inducing skipping of exon 51 of a DMD pre-mRNA in a muscle cell of a subject.
43. Use of a complex in the manufacture of a medicament for treating Duchenne muscular dystrophy in a subject in need thereof, wherein a complex comprising an anti-transferrin receptor antibody covalently linked to an exon-skipping oligonucleotide is to be administered to the subject,
- wherein the anti-transferrin receptor antibody comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region and the light chain variable region comprise human or humanized framework regions; wherein the anti-transferrin receptor antibody binds in the range of C89 to F760 of human transferrin receptor protein 1 (TfR1) having an amino acid sequence as set forth in SEQ ID NO: 1;
- wherein the oligonucleotide is 30 nucleotides in length, wherein the oligonucleotide is a phosphorodiamidate morpholino oligomer (PMO) comprising a region of complementarity fully complementary to the annealing site of an oligonucleotide consisting of the sequence of SEQ ID

NO: 195, wherein the nucleobases of the region of complementarity are selected from adenosine, thymidine, guanosine, and cytosine; and

wherein the oligonucleotide is covalently linked at the 5' end to a lysine residue of the anti-transferrin receptor antibody via a cleavable linker comprising a valine-citrulline sequence.

44. The use according to claim 43, wherein the cleavable linker comprises a triazole covalently linked to the valine-citrulline sequence and further covalently linked to a lysine residue of the anti-transferrin receptor antibody,

wherein the triazole is obtained by a cycloaddition reaction between an azide and an alkyne to form the triazole, and

wherein prior to the cycloaddition reaction, the azide is covalently linked to the valine-citrulline sequence of the cleavable linker that is covalently linked to the 5' end of the oligonucleotide and the alkyne is provided in a bicyclononyne moiety.

45. The use according to claim 43 or claim 44, wherein the anti-transferrin receptor antibody is not glycosylated.

46. The use according to any one of claims 43 to 45, wherein the oligonucleotide of the complex promotes the expression or activity of a functional dystrophin protein in a muscle cell of the subject.

47. The use according to claim 46, wherein the muscle cell is a skeletal muscle cell or a cardiac muscle cell.

48. The use according to any one of claims 43 to 47, wherein the subject is human.

49. The use according to any one of claims 43 to 48, wherein the complex is to be administered to the subject intravenously.