MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING DYSTROPHINOPATHIES

RELATED APPLICATIONS

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 63/143829, entitled "MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING DYSTROPHINOPATHIES", filed on January 30, 2021, to U.S. Provisional Application Serial No. 63/069077, entitled "MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING DYSTROPHINOPATHIES", filed on August 23, 2020, and to U.S. Provisional Application Serial No. 63/055777, entitled "MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING DYSTROPHINOPATHIES", filed on July 23, 2020; the contents of each of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present application relates to targeting complexes for delivering molecular payloads (e.g., oligonucleotides) to cells and uses thereof, particularly uses relating to treatment of disease.

REFERENCE TO SEQUENCE LISTING SUBMITTED AS A TEXT FILE VIA EFS-WEB

[0003] The instant application contains a sequence listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 8, 2021, is named D082470040WO00-SEQ-DWY and is 575,156 bytes in size.

BACKGROUND OF INVENTION

[0004] Dystrophinopathies are a group of distinct neuromuscular diseases that result from mutations in dystrophin gene. Dystrophinopathies include Duchenne muscular dystrophy, Becker muscular dystrophy, and X-linked dilated cardiomyopathy. Dystrophin (DMD) is a large gene, containing 79 exons and about 2.6 million total base pairs. Numerous mutations in DMD, including exonic frameshift, deletion, substitution, and duplicative mutations, are able to diminish the expression of functional dystrophin, leading to

dystrophinopathies. One agent that targets exon 51 of human DMD, eteplirsen, has been preliminarily approved by the U.S. Food and Drug Administration (FDA) however its efficacy is still being evaluated.

SUMMARY OF INVENTION

[0005] According to some aspects, the disclosure provides complexes that target muscle cells for purposes of delivering molecular payloads to those cells. In some embodiments, complexes provided herein are particularly useful for delivering molecular payloads that increase or restore expression or activity of functional DMD. In some embodiments, complexes comprise oligonucleotide based molecular payloads that promote normal expression of functional DMD through an in-frame exon skipping mechanism or suppression of stop codons. In other embodiments, complexes are configured for delivering a mini-dystrophin gene or synthetic mRNA that increases or restores functional dystrophin activity. Accordingly, in some embodiments, complexes provided herein comprise muscle-targeting agents (e.g., muscle targeting antibodies) that specifically bind to receptors on the surface of muscle cells for purposes of delivering molecular payloads to the muscle cells. In some embodiments, the complexes are taken up into the cells via a receptor mediated internalization, following which the molecular payload may be released to perform a function inside the cells. For example, complexes engineered to deliver oligonucleotides may release the oligonucleotides such that the oligonucleotides can promote expression of functional DMD (e.g., through an exon skipping mechanism) in the muscle cells. In some embodiments, the oligonucleotides are released by endosomal cleavage of covalent linkers connecting oligonucleotides and muscle-targeting agents of the complexes.

[0006] One aspect of the present disclosure relates to a complex comprising an anti-transferrin receptor (TfR) antibody covalently linked to a molecular payload configured for promoting the expression or activity of a DMD gene, wherein the antibody comprises:

(i) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 76; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 75;

(ii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 69; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 70;

(iii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 71; and/or a light chain variable region (VL) comprising an

amino acid sequence at least 95% identical to SEQ ID NO: 70;

(iv) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 72; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 70;

(v) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 73; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 74;

(vi) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 73; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 75;

(vii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 76; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 74;

(viii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 77; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 78;

(ix) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 79; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 80; or

(x) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 77; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 80.

[0007] In some embodiments, the antibody comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 76 and a VL comprising the amino acid sequence of SEQ ID NO: 75;

(ii) a VH comprising the amino acid sequence of SEQ ID NO: 69 and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(iii) a VH comprising the amino acid sequence of SEQ ID NO: 71and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(iv) a VH comprising the amino acid sequence of SEQ ID NO: 72 and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(v) a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of SEQ ID NO: 74;

(vi) a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL

comprising the amino acid sequence of SEQ ID NO: 75;

(vii) a VH comprising the amino acid sequence of SEQ ID NO: 76 and a VL comprising the amino acid sequence of SEQ ID NO: 74;

(viii) a VH comprising the amino acid sequence of SEQ ID NO: 77 and a VL comprising the amino acid sequence of SEQ ID NO: 78;

(ix) a VH comprising the amino acid sequence of SEQ ID NO: 79 and a VL comprising the amino acid sequence of SEQ ID NO: 80; or

(x) a VH comprising the amino acid sequence of SEQ ID NO: 77 and a VL comprising the amino acid sequence of SEQ ID NO: 80.

[0008] In some embodiments, the antibody is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a scFv, a Fv, and a full-length IgG. In some embodiments, the antibody is a Fab fragment.

[0009] In some embodiments, the antibody comprises:

(i) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 101; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 90;

(ii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 97; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(iii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 98; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(iv) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 99; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(v) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 100; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 89;

(vi) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 100; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 90;

(vii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 101; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 89;

(viii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 102; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 93;

(ix) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 103; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 95; or

(x) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 102; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 95.

[00010] In some embodiments, the antibody comprises:

(i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 101; and a light chain comprising the amino acid sequence of SEQ ID NO: 90;

(ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 97; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(iii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 98; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(iv) a heavy chain comprising the amino acid sequence of SEQ ID NO: 99; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(v) a heavy chain comprising the amino acid sequence of SEQ ID NO: 100; and a light chain comprising the amino acid sequence of SEQ ID NO: 89;

(vi) a heavy chain comprising the amino acid sequence of SEQ ID NO: 100; and a light chain comprising the amino acid sequence of SEQ ID NO: 90;

(vii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 101; and a light chain comprising the amino acid sequence of SEQ ID NO: 89;

(viii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 102; and a light chain comprising the amino acid sequence of SEQ ID NO: 93;

(ix) a heavy chain comprising the amino acid sequence of SEQ ID NO: 103; and a light chain comprising the amino acid sequence of SEQ ID NO: 95; or

(x) a heavy chain comprising the amino acid sequence of SEQ ID NO: 102; and a light chain comprising the amino acid sequence of SEQ ID NO: 95.

[00011] In some embodiments, the antibody does not specifically bind to the transferrin binding site of the transferrin receptor and/or the muscle-targeting antibody does not inhibit binding of transferrin to the transferrin receptor.

[00012] In some embodiments, the molecular payload is an oligonucleotide. In some embodiments, the oligonucleotide promotes exon skipping in a DMD RNA. In some embodiments, the oligonucleotide promotes skipping of an exon of DMD in the range of exon 8 to exon 55. In some embodiments, the oligonucleotide promotes skipping of exon 8, exon 23, exon 43, exon 44, exon 45, exon 46, exon 50, exon 51, exon 52, exon 53, and/or exon 55.

[00013] In some embodiments, the oligonucleotide comprises a region of complementarity to one or more full or partial exonic splicing enhancers (ESE) of a DMD transcript. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 402-436 and 2043-2238.

[00014] In some embodiments, the oligonucleotide promotes skipping of exon 51.

[00015] In some embodiments, the oligonucleotide is 20-30 nucleotides in length and comprises a region of complementarity to a target sequence comprising at least 4 consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 402-436.

[00016] In some embodiments, the oligonucleotide comprises any one of SEQ ID NOs: 437-1241, or comprises a region of complementarity to any one of SEQ ID NOs: 1242-2046.

[00017] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence of an oligonucleotide listed in Table 14. In some embodiments, the oligonucleotide comprises a sequence listed in Table 14, wherein any one or more of the uracil bases (U's) in the oligonucleotide may optionally be a thymine base (T).

[00018] In some embodiments, the oligonucleotide comprises at least one modified internucleoside linkage. In some embodiments, the at least one modified internucleoside linkage is a phosphorothioate linkage.

[00019] In some embodiments, the oligonucleotide comprises one or more modified nucleosides. In some embodiments, the one or more modified nucleosides are 2'-modified nucleosides.

[00020] In some embodiments, the oligonucleotide comprises one or more phosphorodiamidate morpholinos, optionally wherein the oligonucleotide is a phosphorodiamidate morpholino oligomer (PMO).

[00021] In some embodiments, the antibody is covalently linked to the molecular payload via a cleavable linker. In some embodiments, the cleavable linker comprises a valine-citrulline sequence.

[00022] In some embodiments, the antibody is covalently linked to the molecular payload via conjugation to a lysine residue or a cysteine residue of the antibody.

[00023] Another aspect of the present disclosure relates to a method of promoting the expression or activity of a DMD protein in a cell, the method comprising contacting the cell with a complex disclosed herein in an amount effective for promoting internalization of the molecular payload to the cell, optionally wherein the cell is a muscle cell.

[00024] Another aspect of the present disclosure relates to a method of treating a subject having a mutated DMD allele that is associated with a dystrophinopathy, the method comprising administering to the subject an effective amount of a complex disclosed herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[00025] **FIG. 1** depicts a non-limiting schematic showing the effect of transfecting cells with an siRNA.

[00026] **FIG. 2** depicts a non-limiting schematic showing the activity of a muscle targeting complex comprising an siRNA.

[00027] **FIGs. 3A-3B** depict non-limiting schematics showing the activity of a muscle targeting complex comprising an siRNA in mouse muscle tissues (gastrocnemius and heart) in vivo, relative to control non-targeting complex comprising the same siRNA. (N=4 C57BL/6 WT mice)

[00028] **FIGs. 4A-4E** depict non-limiting schematics showing the tissue selectivity of a muscle targeting complex comprising an siRNA.

[00029] **FIG. 5 depicts** a non-limiting schematic showing the ability of an anti-transferrin receptor muscle targeting complex comprising an exon-23 skipping phosphorodiamidate morpholino oligomer (PMO) to dose-dependently enhance exon skipping in muscle tissues of a *mdx* mouse model.

[00030] **FIGs. 6A-6B** depict non-limiting schematics showing the ability of an anti-transferrin receptor muscle targeting complex comprising an exon-23 skipping PMO to dose-dependently increase dystrophin in skeletal muscle (quadriceps) of a *mdx* mouse model.

[00031] **FIGs. 7A-7E** depict non-limiting schematics showing the ability of an anti-transferrin receptor muscle targeting complex comprising an exon-23 skipping PMO to improve functional performance (FIGs. 7A, 7B, 7C, and 7D) and reduce creatine kinase levels (FIG. 7E) in an *mdx* mouse model. (** $p < 0.01$; *** $p < 0.001$; ****; $p < 0.0001$; NS not significant)

[00032] **FIG. 8** shows the serum stability of the linker used for linking an anti-TfR antibody and a molecular payload (e.g., an oligonucleotide) in various species over time after intravenous administration.

[00033] **FIGs. 9A-9F** show binding of humanized anti-TfR Fabs to human TfR1 (hTfR1) or cynomolgus monkey TfR1 (cTfR1), as measured by ELISA. FIG. 9A shows binding of humanized 3M12 variants to hTfR1. FIG. 9B shows binding of humanized 3M12 variants to cTfR1. FIG. 9C shows binding of humanized 3A4 variants to hTfR1. FIG. 9D shows binding of humanized 3A4 variants to cTfR1. FIG. 9E shows binding of humanized 5H12 variants to hTfR1. FIG. 9F shows binding of humanized 5H12 variants to hTfR1.

[00034] **FIGs. 10** shows the quantified cellular uptake of anti-TfR Fab conjugates into rhabdomyosarcoma (RD) cells. The molecular payload in the tested conjugates are DMPK-targeting oligonucleotides and the uptake of the conjugates were facilitated by indicated anti-TfR Fabs. Conjugates having a negative control Fab (anti-mouse TfR) or a positive control Fab (anti-human TfR1) are also included this assay. Cells were incubated with indicated conjugate at a concentration of 100 nM for 4 hours. Cellular uptake was measured by mean Cypher5e fluorescence.

[00035] **FIGs. 11A-11F** show binding of oligonucleotide-conjugated or unconjugated humanized anti-TfR Fabs to human TfR1 (hTfR1) and cynomolgus monkey TfR1 (cTfR1), as measured by ELISA. FIG. 11A shows the binding of humanized 3M12 variants alone or in conjugates with a DMPK targeting oligo to hTfR1. FIG. 11B shows the binding of humanized 3M12 variants alone or in conjugates with a DMPK targeting oligo to cTfR1. FIG. 11C shows the binding of humanized 3A4 variants alone or in conjugates with a DMPK targeting oligo to hTfR1. FIG. 11D shows the binding of humanized 3A4 variants alone or in conjugates with a DMPK targeting oligo to cTfR1. FIG. 11E shows the binding of humanized 5H12 variants alone or in conjugates with a DMPK targeting oligo to hTfR1. FIG. 11F shows the binding of humanized 5H12 variants alone or in conjugates with a DMPK targeting oligo to cTfR1. The respective EC_{50} values are also shown.

[00036] **FIG. 12** shows DMPK expression in RD cells treated with various concentrations of conjugates containing the indicated humanized anti-TfR antibodies conjugated to a DMPK-targeting oligonucleotide ASO300. The duration of treatment was 3 days. ASO300 delivered using transfection agents were used as control.

[00037] **FIG. 13** shows skipping of exon 51 in human DMD myotubes, facilitated by a DMD exon 51 skipping oligonucleotide (a PMO). Cells were treated with the naked PMO or with PMO conjugated to an anti-TfR1 Fab (Ab-PMO).

[00038] **FIG. 14** shows dose-dependent increase of dystrophin expression in quadriceps muscles of mdx mice after treatment with anti-mouse TfR1 (RI7 217) conjugated to an oligonucleotide (a PMO) targeted to exon 23, as measured by western blotting for dystrophin,

with alpha-actin as a loading control. The standards were generated using pooled wild-type protein and pooled mdx protein. The percent indicates the amount of WT protein spiked into the sample.

[00039] **FIG. 15** shows quantification of dystrophin protein levels within quadriceps muscles of *mdx* mice after treatment with various doses of anti-mouse TfR (RI7 217) conjugated to an oligonucleotide (a PMO) targeting exon 23.

[00040] **FIG. 16** shows immunofluorescent staining images of quadriceps muscles from wild-type (WT) mice treated with saline, or *mdx* mice treated with saline, naked oligonucleotide or oligonucleotide conjugated to anti- mouse TfR1 (RI7 217).

[00041] **FIG. 17** shows data illustrating that conjugates containing designated anti-TfR Fabs (3M12 VH3/VK2, 3M12 VH4/VK3, and 3A4 VH3 N54S/VK4) conjugated to a DMD exon-skipping oligonucleotide resulted in enhanced exon skipping compared to the naked DMD exon skipping oligo in DMD patient myotubes.

[00042] **FIG. 18** shows ELISA measurements of binding of anti-TfR Fab 3M12 VH4/Vk3 to recombinant human (circles), cynomolgus monkey (squares), mouse (upward triangles), or rat (downward triangles) TfR1 protein, at a range of concentrations from 230 pM to 500 nM of the Fab. Measurement results show that the anti-TfR Fab is reactive with human and cynomolgus monkey TfR1. Binding was not observed to mouse or rat recombinant TfR1. Data is shown as relative fluorescent units normalized to baseline.

[00043] **FIG. 19** shows results of an ELISA testing the affinity of anti-TfR Fab 3M12 VH4/Vk3 to recombinant human TfR1 or TfR2 over a range of concentrations from 230 pM to 500 nM of Fab. The data are presented as relative fluorescence units normalized to baseline. The results demonstrate that the Fab does not bind recombinant human TfR2.

[00044] **FIG. 20** shows the serum stability of the linker used for linking anti-TfR Fab 3M12 VH4/Vk3 to a control antisense oligonucleotide over 72 hours incubation in PBS or in rat, mouse, cynomolgus monkey or human serum.

[00045] **FIGs. 21A-21C** show quantification of exon 23 skipping in quadriceps (**FIG. 21A**), heart (**FIG. 21B**), and diaphragm (**FIG. 21C**) of wild-type (WT) and *mdx* mice two- or four-weeks following administration of a single dose of saline, unconjugated oligonucleotide (ASO) that induces exon 23 skipping in DMD, or conjugates containing an anti-TfR RI7217 Fab conjugated to the ASO (Ab-ASO). Little or no exon 23 skipping was observed in tissues from WT mice or from *mdx* mice administered saline or unconjugated ASO, whereas significant levels of exon 23 skipping was observed in tissues of *mdx* mice treated with Ab-ASO. (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$)

[00046] **FIGs. 22A-22D** show measurement of dystrophin protein in quadriceps of *mdx* mice following administration of a single dose of unconjugated oligonucleotide (ASO) that induces exon 23 skipping in DMD, or conjugates containing an anti-TfR1 RI7217 Fab conjugated to the ASO (Ab-ASO). **FIG. 22A** shows western blots of dystrophin and alpha-actinin protein in muscle tissue two weeks following injection of ASO or Ab-ASO. **FIG. 22B** shows quantification of the dystrophin in the western blot of FIG. 23A relative to dystrophin protein in wild-type muscle. **FIG. 22C** shows western blots of dystrophin and alpha-actinin protein in muscle tissue four weeks following injection of ASO or Ab-ASO. **FIG. 22D** shows quantification of the dystrophin in the western blot of FIG. 22C relative to dystrophin protein in wild-type muscle. The standard curves in FIGs. 22A and 22C were generated by pooling tissue from wild-type (WT) and *mdx* mouse samples, and the percent WT indicates the amount of WT protein spiked into each sample. (* $p < 0.05$; ns, not significant)

[00047] **FIGs. 23A-23D** show measurement of dystrophin protein in heart muscle of *mdx* mice following administration of a single dose of unconjugated oligonucleotide (ASO) that induces exon 23 skipping in DMD, or conjugates containing an anti-TfR1 RI7217 Fab conjugated to the ASO (Ab-ASO). **FIG. 23A** shows western blots of dystrophin and alpha-actinin protein in muscle tissue two weeks following injection of ASO or Ab-ASO. **FIG. 23B** shows quantification of the dystrophin in the western blot of FIG. 23A relative to dystrophin protein in wild-type muscle. **FIG. 23C** shows western blots of dystrophin and alpha-actinin protein in muscle tissue four weeks following injection of ASO or Ab-ASO. **FIG. 23D** shows quantification of the dystrophin in the Western blot of FIG. 23C relative to dystrophin protein in wild-type muscle. The standard curves in FIGs. 23A and 23C were generated by pooling tissue from wild-type (WT) and *mdx* mouse samples, and the percent WT indicates the amount of WT protein spiked into each sample. (* $p < 0.05$, **** $p < 0.0001$)

[00048] **FIGs. 24A-24D** show measurement of dystrophin protein in diaphragm muscle of *mdx* mice following administration of a single dose of unconjugated oligonucleotide (ASO) that induces exon 23 skipping in DMD, or conjugates containing an anti-TfR1 RI7217 Fab conjugated to the ASO (Ab-ASO). **FIG. 24A** shows western blots of dystrophin and alpha-actinin protein in muscle tissue two weeks following injection of ASO or Ab-ASO. **FIG. 24B** shows quantification of the dystrophin in the western blot of FIG. 24A relative to dystrophin protein in wild-type muscle. **FIG. 24C** shows western blots of dystrophin and alpha-actinin protein in muscle tissue four weeks following injection of ASO or Ab-ASO. **FIG. 24D** shows quantification of the dystrophin in the Western blot of FIG. 24C relative to dystrophin protein in wild-type muscle. The standard curves in FIGs. 24A and 24C were generated by pooling

tissue from wild-type (WT) and *mdx* mouse samples, and the percent WT indicates the amount of WT protein spiked into each sample. (** $p < 0.01$, *** $p < 0.001$)

[00049] **FIGs. 25A-25C** show quantification of the amount of administered oligonucleotide (ASO) in quadriceps (**FIG. 25A**), diaphragm (**FIG. 25B**), and heart (**FIG. 25C**) of wild-type (WT) or *mdx* mice two- or four-weeks following administration of a single dose of saline, unconjugated exon 23 skipping oligonucleotide (ASO), or conjugates containing an anti-TfR1 RI7217 Fab conjugated to the ASO (Ab-ASO).

[00050] **FIG. 26** shows % exon 53 skipping in DMD patient cells harboring a deletion of DMD exon 52, following gymnotic uptake of exon 53-skipping oligonucleotides over a range of concentrations.

[00051] **FIG. 27** shows % exon 53 skipping in DMD patient cells harboring a deletion of DMD exon 52, following treatment with exon 53-skipping PMO either not linked to an antibody ("Naked ASO") or covalently linked to an anti-TfR1 Fab ("Anti-TfR1 Fab-ASO complex") at a variety of concentrations.

DETAILED DESCRIPTION OF INVENTION

[00052] Aspects of the disclosure relate to a recognition that while certain molecular payloads (*e.g.,* oligonucleotides, peptides, small molecules) can have beneficial effects in muscle cells, it has proven challenging to effectively target such cells. As described herein, the present disclosure provides complexes comprising muscle-targeting agents covalently linked to molecular payloads in order to overcome such challenges. In some embodiments, the complexes are particularly useful for delivering molecular payloads that modulate (e.g., promote) the expression or activity of target genes in muscle cells, e.g., in a subject having or suspected of having a rare muscle disease. For example, in some embodiments, complexes are provided for targeting DMD, *e.g.,* a mutated DMD allele. In some embodiments, complexes provided herein may comprise oligonucleotides that promote normal expression and activity of DMD. As another example, complexes may comprise oligonucleotides that induce skipping of exon of DMD mRNA. In some embodiments, synthetic nucleic acid payloads (*e.g.,* DNA or RNA payloads) may be used that express one or more proteins that promote normal expression and activity of DMD.

[00053] In some embodiments, complexes may comprise molecular payloads of synthetic cDNAs and/or (e.g., and) synthetic mRNAs, e.g., that express dystrophin or fragments thereof (e.g., a dystrophin mini gene). In some embodiments, complexes may

comprise molecular payloads such as guide molecules (*e.g.,* guide RNAs) that are capable of targeting nucleic acid programmable nucleases (*e.g.*, Cas9) to a sequence at or near a disease-associated mutation of DMD, *e.g.,* a mutated DMD exon. In some embodiments, such nucleic programmable nucleases could be used to cleave part or all of a disease-associated mutation of DMD, *e.g.,* a mutated DMD exon, to promote expression of functional DMD. In some embodiments, complexes may comprise molecular payloads that upregulate the expression and/or (e.g., and) activity of genes that can replace the function of dystrophin, such as utrophin.

[00054] Further aspects of the disclosure, including a description of defined terms, are provided below.

I. Definitions

[00055] **Administering**: As used herein, the terms "administering" or "administration" means to provide a complex to a subject in a manner that is physiologically and/or (e.g., and) pharmacologically useful (*e.g.*, to treat a condition in the subject).

[00056] **Approximately:** As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[00057] **Antibody:** As used herein, the term "antibody" refers to a polypeptide that includes at least one immunoglobulin variable domain or at least one antigenic determinant, e.g., paratope that specifically binds to an antigen. In some embodiments, an antibody is a full-length antibody. In some embodiments, an antibody is a chimeric antibody. In some embodiments, an antibody is a humanized antibody. However, in some embodiments, an antibody is a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a Fv fragment or a scFv fragment. In some embodiments, an antibody is a nanobody derived from a camelid antibody or a nanobody derived from shark antibody. In some embodiments, an antibody is a diabody. In some embodiments, an antibody comprises a framework having a human germline sequence. In another embodiment, an antibody comprises a heavy chain constant domain selected from the group consisting of IgG, IgG1, IgG2, IgG2A, IgG2B, IgG2C, IgG3, IgG4, IgA1, IgA2, IgD, IgM, and IgE constant domains. In some embodiments, an antibody comprises a heavy (H) chain variable region (abbreviated herein as VH), and/or (e.g., and) a

light (L) chain variable region (abbreviated herein as VL). In some embodiments, an antibody comprises a constant domain, e.g., an Fc region. An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences and their functional variations are known. With respect to the heavy chain, in some embodiments, the heavy chain of an antibody described herein can be an alpha (α), delta (Δ), epsilon (ε), gamma (γ) or mu (μ) heavy chain. In some embodiments, the heavy chain of an antibody described herein can comprise a human alpha (α), delta (Δ), epsilon (ε), gamma (γ) or mu (μ) heavy chain. In a particular embodiment, an antibody described herein comprises a human gamma 1 CH1, CH2, and/or (e.g., and) CH3 domain. In some embodiments, the amino acid sequence of the VH domain comprises the amino acid sequence of a human gamma (γ) heavy chain constant region, such as any known in the art. Non-limiting examples of human constant region sequences have been described in the art, e.g., see U.S. Pat. No. 5,693,780 and Kabat E A et al., (1991) supra. In some embodiments, the VH domain comprises an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or at least 99% identical to any of the variable chain constant regions provided herein. In some embodiments, an antibody is modified, e.g., modified via glycosylation, phosphorylation, sumoylation, and/or (e.g., and) methylation. In some embodiments, an antibody is a glycosylated antibody, which is conjugated to one or more sugar or carbohydrate molecules. In some embodiments, the one or more sugar or carbohydrate molecule are conjugated to the antibody via N-glycosylation, O-glycosylation, C-glycosylation, glypiation (GPI anchor attachment), and/or (e.g., and) phosphoglycosylation. In some embodiments, the one or more sugar or carbohydrate molecule are monosaccharides, disaccharides, oligosaccharides, or glycans. In some embodiments, the one or more sugar or carbohydrate molecule is a branched oligosaccharide or a branched glycan. In some embodiments, the one or more sugar or carbohydrate molecule includes a mannose unit, a glucose unit, an N-acetylglucosamine unit, an N-acetylgalactosamine unit, a galactose unit, a fucose unit, or a phospholipid unit. In some embodiments, an antibody is a construct that comprises a polypeptide comprising one or more antigen binding fragments of the disclosure linked to a linker polypeptide or an immunoglobulin constant domain. Linker polypeptides comprise two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Examples of linker polypeptides have been reported (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123). Still further, an antibody may be part of a larger immunoadhesion molecule, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or

peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S. M., et al. (1995) Human Antibodies and Hybridomas 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S. M., et al. (1994) Mol. Immunol. 31:1047-1058).

[00058] **CDR:** As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. A typical antibody molecule comprises a heavy chain variable region (VH) and a light chain variable region (VL), which are usually involved in antigen binding. The VH and VL regions can be further subdivided into regions of hypervariability, also known as "complementarity determining regions" ("CDR"), interspersed with regions that are more conserved, which are known as "framework regions" ("FR"). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The extent of the framework region and CDRs can be precisely identified using methodology known in the art, for example, by the Kabat definition, the IMGT definition, the Chothia definition, the AbM definition, and/or (e.g., and) the contact definition, all of which are well known in the art. See, e.g., Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; IMGT®, the international ImMunoGeneTics information system® http://www.imgt.org, Lefranc, M.-P. et al., Nucleic Acids Res., 27:209-212 (1999); Ruiz, M. et al., Nucleic Acids Res., 28:219-221 (2000); Lefranc, M.-P., Nucleic Acids Res., 29:207-209 (2001); Lefranc, M.-P., Nucleic Acids Res., 31:307-310 (2003); Lefranc, M.-P. et al., In Silico Biol., 5, 0006 (2004) [Epub], 5:45-60 (2005); Lefranc, M.-P. et al., Nucleic Acids Res., 33:D593-597 (2005); Lefranc, M.-P. et al., Nucleic Acids Res., 37:D1006-1012 (2009); Lefranc, M.-P. et al., Nucleic Acids Res., 43:D413-422 (2015); Chothia et al., (1989) Nature 342:877; Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, Al-lazikani et al (1997) J. Molec. Biol. 273:927-948; and Almagro, J. Mol. Recognit. 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs. As used herein, a CDR may refer to the CDR defined by any method known in the art. Two antibodies having the same CDR means that the two antibodies have the same amino acid sequence of that CDR as determined by the same method, for example, the IMGT definition.

[00059] There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term "CDR set" as used herein refers to a group of three CDRs that occur in a single

variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat *et al.*, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Sub-portions of CDRs may be designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H" designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (FASEB J. 9:133-139 (1995)) and MacCallum (J Mol Biol 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems. Examples of CDR definition systems are provided in Table 1.

Table 1. CDR Definitions

	IMGT[1]	Kabat[2]	Chothia[3]
CDR-H1	27-38	31-35	26-32
CDR-H2	56-65	50-65	53-55
CDR-H3	105-116/117	95-102	96-101
CDR-L1	27-38	24-34	26-32
CDR-L2	56-65	50-56	50-52
CDR-L3	105-116/117	89-97	91-96

[1] IMGT®, the international ImMunoGeneTics information system®, imgt.org, Lefranc, M.-P. et al., Nucleic Acids Res., 27:209-212 (1999)

[2] Kabat et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242

[3] Chothia et al., J. Mol. Biol. 196:901-917 (1987))

[00060] **CDR-grafted antibody:** The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or (e.g., and) VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (*e.g.*, CDR3) has been replaced with human CDR sequences.

[00061] **Chimeric antibody:** The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region

sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

[00062] **Complementary:** As used herein, the term "complementary" refers to the capacity for precise pairing between two nucleotides or two sets of nucleotides. In particular, complementary is a term that characterizes an extent of hydrogen bond pairing that brings about binding between two nucleotides or two sets of nucleotides. For example, if a base at one position of an oligonucleotide is capable of hydrogen bonding with a base at the corresponding position of a target nucleic acid (e.g., an mRNA), then the bases are considered to be complementary to each other at that position. Base pairings may include both canonical Watson-Crick base pairing and non-Watson-Crick base pairing (e.g., Wobble base pairing and Hoogsteen base pairing). For example, in some embodiments, for complementary base pairings, adenosine-type bases (A) are complementary to thymidine-type bases (T) or uracil-type bases (U), that cytosine-type bases (C) are complementary to guanosine-type bases (G), and that universal bases such as 3-nitropyrrole or 5-nitroindole can hybridize to and are considered complementary to any A, C, U, or T. Inosine (I) has also been considered in the art to be a universal base and is considered complementary to any A, C, U or T.

[00063] **Conservative amino acid substitution:** As used herein, a "conservative amino acid substitution" refers to an amino acid substitution that does not alter the relative charge or size characteristics of the protein in which the amino acid substitution is made. Variants can be prepared according to methods for altering polypeptide sequence known to one of ordinary skill in the art such as are found in references which compile such methods, e.g. Molecular Cloning: A Laboratory Manual, J. Sambrook, et al., eds., Fourth Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2012, or Current Protocols in Molecular Biology, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

[00064] **Covalently linked:** As used herein, the term "covalently linked" refers to a characteristic of two or more molecules being linked together via at least one covalent bond. In some embodiments, two molecules can be covalently linked together by a single bond, e.g., a disulfide bond or disulfide bridge, that serves as a linker between the molecules. However, in some embodiments, two or more molecules can be covalently linked together via a molecule that serves as a linker that joins the two or more molecules together through multiple covalent

bonds. In some embodiments, a linker may be a cleavable linker. However, in some embodiments, a linker may be a non-cleavable linker.

[00065] **Cross-reactive:** As used herein and in the context of a targeting agent (e.g., antibody), the term "cross-reactive," refers to a property of the agent being capable of specifically binding to more than one antigen of a similar type or class (e.g., antigens of multiple homologs, paralogs, or orthologs) with similar affinity or avidity. For example, in some embodiments, an antibody that is cross-reactive against human and non-human primate antigens of a similar type or class (e.g., a human transferrin receptor and non-human primate transferrin receptor) is capable of binding to the human antigen and non-human primate antigens with a similar affinity or avidity. In some embodiments, an antibody is cross-reactive against a human antigen and a rodent antigen of a similar type or class. In some embodiments, an antibody is cross-reactive against a rodent antigen and a non-human primate antigen of a similar type or class. In some embodiments, an antibody is cross-reactive against a human antigen, a non-human primate antigen, and a rodent antigen of a similar type or class.

[00066] **DMD:** As used herein, the term "DMD" refers to a gene that encodes dystrophin protein, a key component of the dystrophin-glycoprotein complex, which bridges the inner cytoskeleton and the extracellular matrix in muscle cells, particularly muscle fibers. Deletions, duplications, and point mutations in DMD may cause dystrophinopathies, such as Duchenne muscular dystrophy, Becker muscular dystrophy, or cardiomyopathy (e.g., DMD-associated dilated cardiomyopathy). Alternative promoter usage and alternative splicing result in numerous distinct transcript variants and protein isoforms for this gene. In some embodiments, a dystrophin gene may be a human (Gene ID: 1756), non-human primate (e.g., Gene ID: 465559), or rodent gene (e.g., Gene ID: 13405; Gene ID: 24907). In addition, multiple human transcript variants (e.g., as annotated under GenBank RefSeq Accession Numbers: NM_000109.3, NM_004006.2 (SEQ ID NO: 2239), NM_004009.3, NM_004010.3 and NM_004011.3) have been characterized that encode different protein isoforms.

[00067] **DMD allele:** As used herein, the term "DMD allele" refers to any one of alternative forms (e.g., wild-type or mutant forms) of a DMD gene. In some embodiments, a DMD allele may encode for dystrophin that retains its normal and typical functions. In some embodiments, a DMD allele may comprise one or more mutations that results in muscular dystrophy. Common mutations that lead to Duchenne muscular dystrophy involve frameshift, deletion, substitution, and duplicative mutations of one or more of 79 exons present in a dystrophin allele, *e.g.*, exon 8, exon 23, exon 41, exon 44, exon 50, exon 51, exon 52, exon 53, or exon 55. Further examples of DMD mutations are disclosed, for example, in Flanigan KM,

et al., *Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort.* Hum Mutat. 2009 Dec; 30 (12):1657-66, the contents of which are incorporated herein by reference in its entirety.

[00068] **Dystrophinopathy:** As used herein, the term "dystrophinopathy" refers to a muscle disease that results from one or more mutated DMD alleles. Dystrophinopathies include a spectrum of conditions (ranging from mild to severe) that includes Duchenne muscular dystrophy, Becker muscular dystrophy, and DMD-associated dilated cardiomyopathy (DCM). In some embodiments, at one end of the spectrum, dystrophinopathy is phenotypically associated with an asymptomatic increase in serum concentration of creatine phosphokinase (CK) and/or (e.g., and) muscle cramps with myoglobinuria. In some embodiments, at the other end of the spectrum, dystrophinopathy is phenotypically associated with progressive muscle diseases that are generally classified as Duchenne or Becker muscular dystrophy when skeletal muscle is primarily affected and as DMD-associated dilated cardiomyopathy (DCM) when the heart is primarily affected. Symptoms of Duchenne muscular dystrophy include muscle loss or degeneration, diminished muscle function, pseudohypertrophy of the tongue and calf muscles, higher risk of neurological abnormalities, and a shortened lifespan. Duchenne muscular dystrophy is associated with Online Mendelian Inheritance in Man (OMIM) Entry # 310200. Becker muscular dystrophy is associated with OMIM Entry # 300376. Dilated cardiomyopathy is associated with OMIM Entry X# 302045.

[00069] **Exonic splicing enhancer (ESE):** As used herein, the term "exonic splicing enhancer" or "ESE" refers to a nucleic acid sequence motif within an exon of a gene, pre-mRNA, or mRNA that directs or enhances splicing of pre-mRNA into mRNA, e.g., as described in Blencowe et al., Trends Biochem Sci 25, 106-10. (2000), incorporated herein by reference. ESEs may direct or enhance splicing, for example, to remove one or more introns and/or one or more exons from a gene transcript. ESE motifs are typically 6-8 nucleobases in length. SR proteins (e.g., proteins encoded by the gene SRSF1, SRSF2, SRSF3, SRSF4, SRSF5, SRSF6, SRSF7, SRSF8, SRSF9, SRSF10, SRSF11, SRSF12, TRA2A or TRA2B) bind to ESEs through their RNA recognition motif region to facilitate splicing. ESE motifs can be identified through a number of methods, including those described in Cartegni et al., Nucleic Acids Research, 2003, Vol. 31, No. 13, 3568–3571, incorporated herein by reference.

[00070] **Framework:** As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs

(CDR-L1, CDR-L2, and CDR-L3 of light chain and CDR-H1, CDR-H2, and CDR-H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FRs within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub-regions constituting a framework region. Human heavy chain and light chain acceptor sequences are known in the art. In one embodiment, the acceptor sequences known in the art may be used in the antibodies disclosed herein.

[00071] **Human antibody:** The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the disclosure may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[00072] **Humanized antibody:** The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (*e.g.,* a mouse) but in which at least a portion of the VH and/or (e.g., and) VL sequence has been altered to be more "human-like", *i.e.,* more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. In one embodiment, humanized anti-transferrin receptor antibodies and antigen binding portions are provided. Such antibodies may be generated by obtaining murine anti-transferrin receptor monoclonal antibodies using traditional hybridoma technology followed by humanization using *in vitro* genetic engineering, such as those disclosed in Kasaian et al PCT publication No. WO 2005/123126 A2.

[00073] **Internalizing cell surface receptor:** As used herein, the term, "internalizing cell surface receptor" refers to a cell surface receptor that is internalized by cells, e.g., upon external stimulation, e.g., ligand binding to the receptor. In some embodiments, an

internalizing cell surface receptor is internalized by endocytosis. In some embodiments, an internalizing cell surface receptor is internalized by clathrin-mediated endocytosis. However, in some embodiments, an internalizing cell surface receptor is internalized by a clathrin-independent pathway, such as, for example, phagocytosis, macropinocytosis, caveolae- and raft-mediated uptake or constitutive clathrin-independent endocytosis. In some embodiments, the internalizing cell surface receptor comprises an intracellular domain, a transmembrane domain, and/or (e.g., and) an extracellular domain, which may optionally further comprise a ligand-binding domain. In some embodiments, a cell surface receptor becomes internalized by a cell after ligand binding. In some embodiments, a ligand may be a muscle-targeting agent or a muscle-targeting antibody. In some embodiments, an internalizing cell surface receptor is a transferrin receptor.

[00074] **Isolated antibody:** An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds transferrin receptor is substantially free of antibodies that specifically bind antigens other than transferrin receptor). An isolated antibody that specifically binds transferrin receptor complex may, however, have cross-reactivity to other antigens, such as transferrin receptor molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or (e.g., and) chemicals.

[00075] **Kabat numbering:** The terms "Kabat numbering", "Kabat definitions and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e. hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat et al. (1971) Ann. NY Acad, Sci. 190:382-391 and, Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

[00076] **Molecular payload:** As used herein, the term "molecular payload" refers to a molecule or species that functions to modulate a biological outcome. In some embodiments, a molecular payload is linked to, or otherwise associated with a muscle-targeting agent. In some

embodiments, the molecular payload is a small molecule, a protein, a peptide, a nucleic acid, or an oligonucleotide. In some embodiments, the molecular payload functions to modulate the transcription of a DNA sequence, to modulate the expression of a protein, or to modulate the activity of a protein. In some embodiments, the molecular payload is an oligonucleotide that comprises a strand having a region of complementarity to a target gene.

[00077] **Muscle-targeting agent:** As used herein, the term, "muscle-targeting agent," refers to a molecule that specifically binds to an antigen expressed on muscle cells. The antigen in or on muscle cells may be a membrane protein, for example an integral membrane protein or a peripheral membrane protein. Typically, a muscle-targeting agent specifically binds to an antigen on muscle cells that facilitates internalization of the muscle-targeting agent (and any associated molecular payload) into the muscle cells. In some embodiments, a muscle-targeting agent specifically binds to an internalizing, cell surface receptor on muscles and is capable of being internalized into muscle cells through receptor mediated internalization. In some embodiments, the muscle-targeting agent is a small molecule, a protein, a peptide, a nucleic acid (*e.g.*, an aptamer), or an antibody. In some embodiments, the muscle-targeting agent is linked to a molecular payload.

[00078] **Muscle-targeting antibody:** As used herein, the term, "muscle-targeting antibody," refers to a muscle-targeting agent that is an antibody that specifically binds to an antigen found in or on muscle cells. In some embodiments, a muscle-targeting antibody specifically binds to an antigen on muscle cells that facilitates internalization of the muscle-targeting antibody (and any associated molecular payment) into the muscle cells. In some embodiments, the muscle-targeting antibody specifically binds to an internalizing, cell surface receptor present on muscle cells. In some embodiments, the muscle-targeting antibody is an antibody that specifically binds to a transferrin receptor.

[00079] **Oligonucleotide:** As used herein, the term "oligonucleotide" refers to an oligomeric nucleic acid compound of up to 200 nucleotides in length. Examples of oligonucleotides include, but are not limited to, RNAi oligonucleotides (e.g., siRNAs, shRNAs), microRNAs, gapmers, mixmers, phosphorodiamidate morpholinos, peptide nucleic acids, aptamers, guide nucleic acids (e.g., Cas9 guide RNAs), etc. Oligonucleotides may be single-stranded or double-stranded. In some embodiments, an oligonucleotide may comprise one or more modified nucleotides (e.g. 2′-O-methyl sugar modifications, purine or pyrimidine modifications). In some embodiments, an oligonucleotide may comprise one or more modified internucleotide linkage. In some embodiments, an oligonucleotide may comprise one or more phosphorothioate linkages, which may be in the Rp or Sp stereochemical conformation.

[00080] **Recombinant antibody:** The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described in more details in this disclosure), antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H. R., (1997) TIB Tech. 15:62-70; Azzazy H., and Highsmith W. E., (2002) Clin. Biochem. 35:425-445; Gavilondo J. V., and Larrick J. W. (2002) BioTechniques 29:128-145; Hoogenboom H., and Chames P. (2000) Immunology Today 21:371-378), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A., and Green L. L. (2002) Current Opinion in Biotechnology 13:593-597; Little M. et al (2000) Immunology Today 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to in vitro mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo. One embodiment of the disclosure provides fully human antibodies capable of binding human transferrin receptor which can be generated using techniques well known in the art, such as, but not limited to, using human Ig phage libraries such as those disclosed in Jermutus et al., PCT publication No. WO 2005/007699 A2.

[00081] **Region of complementarity:** As used herein, the term "region of complementarity" refers to a nucleotide sequence, e.g., of a oligonucleotide, that is sufficiently complementary to a cognate nucleotide sequence, e.g., of a target nucleic acid, such that the two nucleotide sequences are capable of annealing to one another under physiological conditions (e.g., in a cell). In some embodiments, a region of complementarity is fully complementary to a cognate nucleotide sequence of target nucleic acid. However, in some embodiments, a region of complementarity is partially complementary to a cognate nucleotide sequence of target nucleic acid (e.g., at least 80%, 90%, 95% or 99% complementarity). In some embodiments, a region of complementarity contains 1, 2, 3, or 4 mismatches compared with a cognate nucleotide sequence of a target nucleic acid.

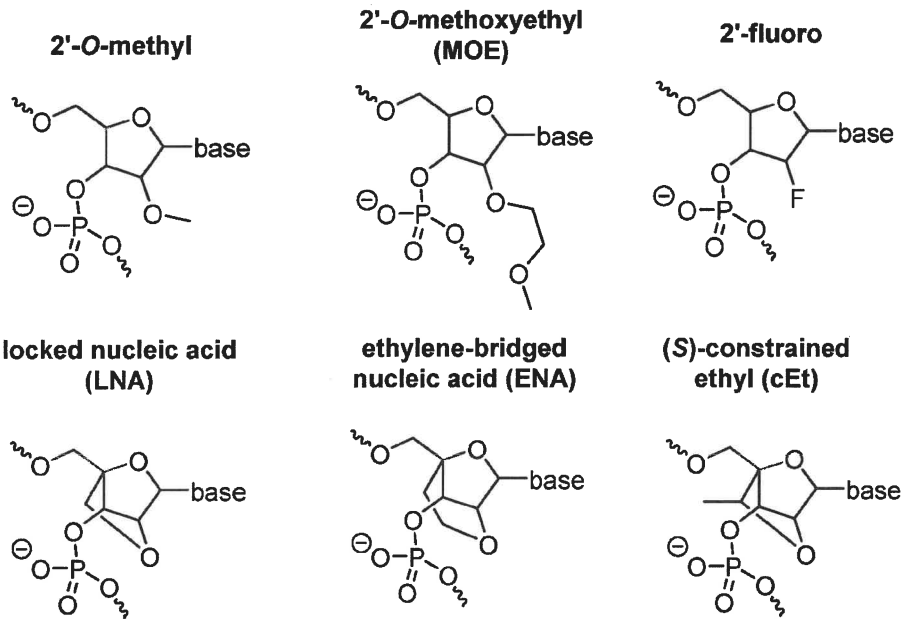
[00082] **Specifically binds:** As used herein, the term "specifically binds" refers to the ability of a molecule to bind to a binding partner with a degree of affinity or avidity that enables the molecule to be used to distinguish the binding partner from an appropriate control in a binding assay or other binding context. With respect to an antibody, the term, "specifically binds", refers to the ability of the antibody to bind to a specific antigen with a degree of affinity or avidity, compared with an appropriate reference antigen or antigens, that enables the antibody to be used to distinguish the specific antigen from others, e.g., to an extent that permits preferential targeting to certain cells, e.g., muscle cells, through binding to the antigen, as described herein. In some embodiments, an antibody specifically binds to a target if the antibody has a K_D for binding the target of at least about 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, or less. In some embodiments, an antibody specifically binds to the transferrin receptor, e.g., an epitope of the apical domain of transferrin receptor.

[00083] **Subject:** As used herein, the term "subject" refers to a mammal. In some embodiments, a subject is non-human primate, or rodent. In some embodiments, a subject is a human. In some embodiments, a subject is a patient, e.g., a human patient that has or is suspected of having a disease. In some embodiments, the subject is a human patient who has or is suspected of having a disease resulting from a mutated DMD gene sequence, e.g., a mutation in an exon of a DMD gene sequence. In some embodiments, a subject has a dystrophinopathy, e.g., Duchenne muscular dystrophy.

[00084] **Transferrin receptor:** As used herein, the term, "transferrin receptor" (also known as TFRC, CD71, p90, TFR, or TFR1) refers to an internalizing cell surface receptor that binds transferrin to facilitate iron uptake by endocytosis. In some embodiments, a transferrin receptor may be of human (NCBI Gene ID 7037), non-human primate (e.g., NCBI Gene ID 711568 or NCBI Gene ID 102136007), or rodent (e.g., NCBI Gene ID 22042) origin. In addition, multiple human transcript variants have been characterized that encoded different isoforms of the receptor (e.g., as annotated under GenBank RefSeq Accession Numbers: NP_001121620.1, NP_003225.2, NP_001300894.1, and NP_001300895.1).

[00085] **2'-modified nucleoside**: As used herein, the terms "2'-modified nucleoside" and "2'-modified ribonucleoside" are used interchangeably and refer to a nucleoside having a sugar moiety modified at the 2' position. In some embodiments, the 2'-modified nucleoside is a 2'-4' bicyclic nucleoside, where the 2' and 4' positions of the sugar are bridged (e.g., via a methylene, an ethylene, or a (S)-constrained ethyl bridge). In some embodiments, the 2'-modified nucleoside is a non-bicyclic 2'-modified nucleoside, e.g., where the 2' position of the

sugar moiety is substituted. Non-limiting examples of 2'-modified nucleosides include: 2'-deoxy, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), 2'-O-N-methylacetamido (2'-O-NMA), locked nucleic acid (LNA, methylene-bridged nucleic acid), ethylene-bridged nucleic acid (ENA), and (S)-constrained ethyl-bridged nucleic acid (cEt). In some embodiments, the 2'-modified nucleosides described herein are high-affinity modified nucleotides and oligonucleotides comprising the 2'-modified nucleotides have increased affinity to a target sequences, relative to an unmodified oligonucleotide. Examples of structures of 2'-modified nucleosides are provided below:



II. Complexes

[00086] Provided herein are complexes that comprise a targeting agent, e.g. an antibody, covalently linked to a molecular payload. In some embodiments, a complex comprises a muscle-targeting antibody covalently linked to an oligonucleotide. A complex may comprise an antibody that specifically binds a single antigenic site or that binds to at least two antigenic sites that may exist on the same or different antigens.

[00087] A complex may be used to modulate the activity or function of at least one gene, protein, and/or (e.g., and) nucleic acid. In some embodiments, the molecular payload present with a complex is responsible for the modulation of a gene, protein, and/or (e.g., and) nucleic acids. A molecular payload may be a small molecule, protein, nucleic acid, oligonucleotide, or any molecular entity capable of modulating the activity or function of a

gene, protein, and/or (e.g., and) nucleic acid in a cell. In some embodiments, a molecular payload is an oligonucleotide that targets a disease-associated repeat in muscle cells.

[00088] In some embodiments, a complex comprises a muscle-targeting agent, e.g. an anti-transferrin receptor antibody, covalently linked to a molecular payload, e.g. a mixmer antisense oligonucleotide that targets a mutated DMD allele to promote exon skipping.

A. Muscle-Targeting Agents

[00089] Some aspects of the disclosure provide muscle-targeting agents, *e.g.*, for delivering a molecular payload to a muscle cell. In some embodiments, such muscle-targeting agents are capable of binding to a muscle cell, *e.g.*, via specifically binding to an antigen on the muscle cell, and delivering an associated molecular payload to the muscle cell. In some embodiments, the molecular payload is bound (*e.g.*, covalently bound) to the muscle targeting agent and is internalized into the muscle cell upon binding of the muscle targeting agent to an antigen on the muscle cell, *e.g.*, via endocytosis. It should be appreciated that various types of muscle-targeting agents may be used in accordance with the disclosure. For example, the muscle-targeting agent may comprise, or consist of, a nucleic acid (*e.g.*, DNA or RNA), a peptide (*e.g.*, an antibody), a lipid (*e.g.*, a microvesicle), or a sugar moiety (*e.g.*, a polysaccharide). Exemplary muscle-targeting agents are described in further detail herein, however, it should be appreciated that the exemplary muscle-targeting agents provided herein are not meant to be limiting.

[00090] Some aspects of the disclosure provide muscle-targeting agents that specifically bind to an antigen on muscle, such as skeletal muscle, smooth muscle, or cardiac muscle. In some embodiments, any of the muscle-targeting agents provided herein bind to (*e.g.*, specifically bind to) an antigen on a skeletal muscle cell, a smooth muscle cell, and/or (e.g., and) a cardiac muscle cell.

[00091] By interacting with muscle-specific cell surface recognition elements (*e.g.*, cell membrane proteins), both tissue localization and selective uptake into muscle cells can be achieved. In some embodiments, molecules that are substrates for muscle uptake transporters are useful for delivering a molecular payload into muscle tissue. Binding to muscle surface recognition elements followed by endocytosis can allow even large molecules such as antibodies to enter muscle cells. As another example molecular payloads conjugated to transferrin or anti-transferrin receptor antibodies can be taken up by muscle cells via binding to transferrin receptor, which may then be endocytosed, *e.g.*, via clathrin-mediated endocytosis.

[00092] The use of muscle-targeting agents may be useful for concentrating a molecular payload (*e.g.*, oligonucleotide) in muscle while reducing toxicity associated with effects in other tissues. In some embodiments, the muscle-targeting agent concentrates a bound molecular payload in muscle cells as compared to another cell type within a subject. In some embodiments, the muscle-targeting agent concentrates a bound molecular payload in muscle cells (*e.g.*, skeletal, smooth, or cardiac muscle cells) in an amount that is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times greater than an amount in non-muscle cells (*e.g.*, liver, neuronal, blood, or fat cells). In some embodiments, a toxicity of the molecular payload in a subject is reduced by at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or 95% when it is delivered to the subject when bound to the muscle-targeting agent.

[00093] In some embodiments, to achieve muscle selectivity, a muscle recognition element (*e.g.*, a muscle cell antigen) may be required. As one example, a muscle-targeting agent may be a small molecule that is a substrate for a muscle-specific uptake transporter. As another example, a muscle-targeting agent may be an antibody that enters a muscle cell via transporter-mediated endocytosis. As another example, a muscle targeting agent may be a ligand that binds to cell surface receptor on a muscle cell. It should be appreciated that while transporter-based approaches provide a direct path for cellular entry, receptor-based targeting may involve stimulated endocytosis to reach the desired site of action.

i. Muscle-Targeting Antibodies

[00094] In some embodiments, the muscle-targeting agent is an antibody. Generally, the high specificity of antibodies for their target antigen provides the potential for selectively targeting muscle cells (*e.g.*, skeletal, smooth, and/or (e.g., and) cardiac muscle cells). This specificity may also limit off-target toxicity. Examples of antibodies that are capable of targeting a surface antigen of muscle cells have been reported and are within the scope of the disclosure. For example, antibodies that target the surface of muscle cells are described in Arahata K., et al. "Immunostaining of skeletal and cardiac muscle surface membrane with antibody against Duchenne muscular dystrophy peptide" *Nature* 1988; 333: 861-3; Song K.S., et al. "Expression of caveolin-3 in skeletal, cardiac, and smooth muscle cells. Caveolin-3 is a component of the sarcolemma and co-fractionates with dystrophin and dystrophin-associated glycoproteins" *J Biol Chem* 1996; 271: 15160-5; and Weisbart R.H. et al., "Cell type specific targeted intracellular delivery into muscle of a monoclonal antibody that binds myosin IIb" *Mol Immunol.* 2003 Mar, 39(13):78309; the entire contents of each of which are incorporated herein by reference.

a. Anti-Transferrin Receptor Antibodies

[00095] Some aspects of the disclosure are based on the recognition that agents binding to transferrin receptor, e.g., anti-transferrin-receptor antibodies, are capable of targeting muscle cell. Transferrin receptors are internalizing cell surface receptors that transport transferrin across the cellular membrane and participate in the regulation and homeostasis of intracellular iron levels. Some aspects of the disclosure provide transferrin receptor binding proteins, which are capable of binding to transferrin receptor. Accordingly, aspects of the disclosure provide binding proteins (*e.g.*, antibodies) that bind to transferrin receptor. In some embodiments, binding proteins that bind to transferrin receptor are internalized, along with any bound molecular payload, into a muscle cell. As used herein, an antibody that binds to a transferrin receptor may be referred to interchangeably as an, transferrin receptor antibody, an anti-transferrin receptor antibody, or an anti-TfR antibody. Antibodies that bind, e.g. specifically bind, to a transferrin receptor may be internalized into the cell, e.g. through receptor-mediated endocytosis, upon binding to a transferrin receptor.

[00096] It should be appreciated that anti-transferrin receptor antibodies may be produced, synthesized, and/or (e.g., and) derivatized using several known methodologies, e.g. library design using phage display. Exemplary methodologies have been characterized in the art and are incorporated by reference (Díez, P. et al. "High-throughput phage-display screening in array format", Enzyme and microbial technology, 2015, 79, 34-41.; Christoph M. H. and Stanley, J.R. "Antibody Phage Display: Technique and Applications" J Invest Dermatol. 2014, 134:2.; Engleman, Edgar (Ed.) "Human Hybridomas and Monoclonal Antibodies." 1985, Springer.). In other embodiments, an anti-transferrin antibody has been previously characterized or disclosed. Antibodies that specifically bind to transferrin receptor are known in the art (see, e.g. US Patent. No. 4,364,934, filed 12/4/1979, "Monoclonal antibody to a human early thymocyte antigen and methods for preparing same"; US Patent No. 8,409,573, filed 6/14/2006, "Anti-CD71 monoclonal antibodies and uses thereof for treating malignant tumor cells"; US Patent No. 9,708,406, filed 5/20/2014, "Anti-transferrin receptor antibodies and methods of use"; US 9,611,323, filed 12/19/2014, "Low affinity blood brain barrier receptor antibodies and uses therefor"; WO 2015/098989, filed 12/24/2014, "Novel anti-Transferrin receptor antibody that passes through blood-brain barrier"; Schneider C. et al. "Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9." J Biol Chem. 1982, 257:14, 8516-8522.; Lee et al. "Targeting Rat Anti-Mouse Transferrin Receptor Monoclonal Antibodies through Blood-Brain Barrier in Mouse" 2000, J Pharmacol. Exp. Ther., 292: 1048-1052.).

[00097] Provided herein, in some aspects, are new anti-TfR antibodies for use as the muscle targeting agents (e.g., in muscle targeting complexes). In some embodiments, the anti-TfR antibody described herein binds to transferrin receptor with high specificity and affinity. In some embodiments, the anti-TfR antibody described herein specifically binds to any extracellular epitope of a transferrin receptor or an epitope that becomes exposed to an antibody. In some embodiments, anti-TfR antibodies provided herein bind specifically to transferrin receptor from human, non-human primates, mouse, rat, *etc*. In some embodiments, anti-TfR antibodies provided herein bind to human transferrin receptor. In some embodiments, the anti-TfR antibody described herein binds to an amino acid segment of a human or non-human primate transferrin receptor, as provided in SEQ ID NOs: 105-108. In some embodiments, the anti-TfR antibody described herein binds to an amino acid segment corresponding to amino acids 90-96 of a human transferrin receptor as set forth in SEQ ID NO: 105, which is not in the apical domain of the transferrin receptor.

[00098] An example human transferrin receptor amino acid sequence, corresponding to NCBI sequence NP_003225.2 (transferrin receptor protein 1 isoform 1, homo sapiens) is as follows:

MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAVDEEENADNNT
KANVTKPKRCSGSICYGTIAVIVFFLIGFMIGYLGYCKGVEPKTECERLAGTESPVREEP
GEDFPAARRLYWDDLKRKLSEKLDSTDFTGTIKLLNENSYVPREAGSQKDENLALYV
ENQFREFKLSKVWRDQHFVKIQVKDSAQNSVIIVDKNGRLVYLVENPGGYVAYSKAA
TVTGKLVHANFGTKKDFEDLYTPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMD
QTKFPIVNAELSFFGHAHLGTGDPYTPGFPSFNHTQFPPSRSSGLPNIPVQTISRAAAEK
LFGNMEGDCPSDWKTDSTCRMVTSESKNVKLTVSNVLKEIKILNIFGVIKGFVEPDHY
VVVGAQRDAWGPGAAKSGVGTALLLKLAQMFSDMVLKDGFQPSRSIIFASWSAGDF
GSVGATEWLEGYLSSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQNVKHP
VTGQFLYQDSNWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTY
KELIERIPELNKVARAAAEVAGQFVIKLTHDVELNLDYERYNSQLLSFVRDLNQYRAD
IKEMGLSLQWLYSARGDFFRATSRLTTDFGNAEKTDRFVMKKLNDRVMRVEYHFLSP
YVSPKESPFRHVFWGSGSHTLPALLENLKLRKQNNGAFNETLFRNQLALATWTIQGA
ANALSGDVWDIDNEF (SEQ ID NO: 105).

[00099] An example non-human primate transferrin receptor amino acid sequence, corresponding to NCBI sequence NP_001244232.1(transferrin receptor protein 1, Macaca mulatta) is as follows:

MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLGVDEEENTDNNTKPNG
TKPKRCGGNICYGTIAVIIFFLIGFMIGYLGYCKGVEPKTECERLAGTESPAREEPEEDFP
AAPRLYWDDLKRKLSEKLDTTDFTSTIKLLNENLYVPREAGSQKDENLALYIENQFRE
FKLSKVWRDQHFVKIQVKDSAQNSVIIVDKNGGLVYLVENPGGYVAYSKAATVTGK
LVHANFGTKKDFEDLDSPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPI
VKADLSFFGHAHLGTGDPYTPGFPSFNHTQFPPSQSSGLPNIPVQTISRAAAEKLFGNM
EGDCPSDWKTDSTCKMVTSENKSVKLTVSNVLKETKILNIFGVIKGFVEPDHYVVVGA
QRDAWGPGAAKSSVGTALLLKLAQMFSDMVLKDGFQPSRSIIFASWSAGDFGSVGAT
EWLEGYLSSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQDVKHPVTGRSL
YQDSNWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELVERI
PELNKVARAAAEVAGQFVIKLTHDTELNLDYERYNSQLLLFLRDLNQYRADVKEMGL
SLQWLYSARGDFFRATSRLTTDFRNAEKRDKFVMKKLNDRVMRVEYYFLSPYVSPKE
SPFRHVFWGSGSHTLSALLESLKLRRQNNSAFNETLFRNQLALATWTIQGAANALSGD
VWDIDNEF

(SEQ ID NO: 106)

[000100] An example non-human primate transferrin receptor amino acid sequence, corresponding to NCBI sequence XP_005545315.1 (transferrin receptor protein 1, Macaca fascicularis) is as follows:

MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLGVDEEENTDNNTKANG
TKPKRCGGNICYGTIAVIIFFLIGFMIGYLGYCKGVEPKTECERLAGTESPAREEPEEDFP
AAPRLYWDDLKRKLSEKLDTTDFTSTIKLLNENLYVPREAGSQKDENLALYIENQFRE
FKLSKVWRDQHFVKIQVKDSAQNSVIIVDKNGGLVYLVENPGGYVAYSKAATVTGK
LVHANFGTKKDFEDLDSPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPI
VKADLSFFGHAHLGTGDPYTPGFPSFNHTQFPPSQSSGLPNIPVQTISRAAAEKLFGNM
EGDCPSDWKTDSTCKMVTSENKSVKLTVSNVLKETKILNIFGVIKGFVEPDHYVVVGA
QRDAWGPGAAKSSVGTALLLKLAQMFSDMVLKDGFQPSRSIIFASWSAGDFGSVGAT
EWLEGYLSSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQDVKHPVTGRSL
YQDSNWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELVERI
PELNKVARAAAEVAGQFVIKLTHDTELNLDYERYNSQLLLFLRDLNQYRADVKEMGL
SLQWLYSARGDFFRATSRLTTDFRNAEKRDKFVMKKLNDRVMRVEYYFLSPYVSPKE
SPFRHVFWGSGSHTLSALLESLKLRRQNNSAFNETLFRNQLALATWTIQGAANALSGD
VWDIDNEF (SEQ ID NO: 107).

[000101] An example mouse transferrin receptor amino acid sequence, corresponding to NCBI sequence NP_001344227.1 (transferrin receptor protein 1, mus musculus) is as follows:

MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAADEEENADNNMKASV
RKPKRFNGRLCFAAIALVIFFLIGFMSGYLGYCKRVEQKEECVKLAETEETDKSETMET
EDVPTSSRLYWADLKTLLSEKLNSIEFADTIKQLSQNTYTPREAGSQKDESLAYYIENQ
FHEFKFSKVWRDEHYVKIQVKSSIGQNMVTIVQSNGNLDPVESPEGYVAFSKPTEVSG
KLVHANFGTKKDFEELSYSVNGSLVIVRAGEITFAEKVANAQSFNAIGVLIYMDKNKF
PVVEADLALFGHAHLGTGDPYTPGFPSFNHTQFPPSQSSGLPNIPVQTISRAAAEKLFG
KMEGSCPARWNIDSSCKLELSQNQNVKLIVKNVLKERRILNIFGVIKGYEEPDRYVVV
GAQRDALGAGVAAKSSVGTGLLLKLAQVFSDMISKDGFRPSRSIIFASWTAGDFGAVG
ATEWLEGYLSSLHLKAFTYINLDKVVLGTSNFKVSASPLLYTLMGKIMQDVKHPVDG
KSLYRDSNWISKVEKLSFDNAAYPFLAYSGIPAVSFCFCEDADYPYLGTRLDTYEALT
QKVPQLNQMVRTAAEVAGQLIIKLTHDVELNLDYEMYNSKLLSFMKDLNQFKTDIRD
MGLSLQWLYSARGDYFRATSRLTTDFHNAEKTNRFVMREINDRIMKVEYHFLSPYVS
PRESPFRHIFWGSGSHTLSALVENLKLRQKNITAFNETLFRNQLALATWTIQGVANALS
GDIWNIDNEF
(SEQ ID NO: 108)

[000102] In some embodiments, an anti-transferrin receptor antibody binds to an amino acid segment of the receptor as follows:
FVKIQVKDSAQNSVIIVDKNGRLVYLVENPGGYVAYSKAATVTGKLVHANFGTKKDF
EDLYTPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVNAELSFFGHAH
LGTGDPYTPGFPSFNHTQFPPSRSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWKTDS
TCRMVTSESKNVKLTVSNVLKE (SEQ ID NO: 109) and does not inhibit the binding interactions between transferrin receptors and transferrin and/or (e.g., and) human hemochromatosis protein (also known as HFE). In some embodiments, the anti-transferrin receptor antibody described herein does not bind an epitope in SEQ ID NO: 109.

[000103] Appropriate methodologies may be used to obtain and/or (e.g., and) produce antibodies, antibody fragments, or antigen-binding agents, e.g., through the use of recombinant DNA protocols. In some embodiments, an antibody may also be produced through the generation of hybridomas (see, e.g., Kohler, G and Milstein, C. "Continuous cultures of fused cells secreting antibody of predefined specificity" Nature, 1975, 256: 495-497). The antigen-of-interest may be used as the immunogen in any form or entity, e.g., recombinant or a naturally occurring form or entity. Hybridomas are screened using standard methods, e.g. ELISA screening, to find at least one hybridoma that produces an antibody that targets a particular antigen. Antibodies may also be produced through screening of protein expression libraries that express antibodies, e.g., phage display libraries. Phage display library design may

also be used, in some embodiments, (see, e.g. U.S. Patent No 5,223,409, filed 3/1/1991, "Directed evolution of novel binding proteins"; WO 1992/18619, filed 4/10/1992, "Heterodimeric receptor libraries using phagemids"; WO 1991/17271, filed 5/1/1991, "Recombinant library screening methods"; WO 1992/20791, filed 5/15/1992, "Methods for producing members of specific binding pairs"; WO 1992/15679, filed 2/28/1992, and "Improved epitope displaying phage"). In some embodiments, an antigen-of-interest may be used to immunize a non-human animal, e.g., a rodent or a goat. In some embodiments, an antibody is then obtained from the non-human animal, and may be optionally modified using a number of methodologies, e.g., using recombinant DNA techniques. Additional examples of antibody production and methodologies are known in the art (see, e.g. Harlow et al. "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory, 1988.).

[000104] In some embodiments, an antibody is modified, e.g., modified via glycosylation, phosphorylation, sumoylation, and/or (e.g., and) methylation. In some embodiments, an antibody is a glycosylated antibody, which is conjugated to one or more sugar or carbohydrate molecules. In some embodiments, the one or more sugar or carbohydrate molecule are conjugated to the antibody via N-glycosylation, O-glycosylation, C-glycosylation, glypiation (GPI anchor attachment), and/or (e.g., and) phosphoglycosylation. In some embodiments, the one or more sugar or carbohydrate molecules are monosaccharides, disaccharides, oligosaccharides, or glycans. In some embodiments, the one or more sugar or carbohydrate molecule is a branched oligosaccharide or a branched glycan. In some embodiments, the one or more sugar or carbohydrate molecule includes a mannose unit, a glucose unit, an N-acetylglucosamine unit, an N-acetylgalactosamine unit, a galactose unit, a fucose unit, or a phospholipid unit. In some embodiments, there are about 1-10, about 1-5, about 5-10, about 1-4, about 1-3, or about 2 sugar molecules. In some embodiments, a glycosylated antibody is fully or partially glycosylated. In some embodiments, an antibody is glycosylated by chemical reactions or by enzymatic means. In some embodiments, an antibody is glycosylated in vitro or inside a cell, which may optionally be deficient in an enzyme in the N- or O- glycosylation pathway, e.g. a glycosyltransferase. In some embodiments, an antibody is functionalized with sugar or carbohydrate molecules as described in International Patent Application Publication WO2014065661, published on May 1, 2014, entitled, "*Modified antibody, antibody-conjugate and process for the preparation thereof*".

[000105] In some embodiments, the anti-TfR antibody of the present disclosure comprises a VL domain and/or (e.g., and) VH domain of any one of the anti-TfR antibodies selected from Table 2, and comprises a constant region comprising the amino acid sequences

of the constant regions of an IgG, IgE, IgM, IgD, IgA or IgY immunoglobulin molecule, any class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or any subclass (e.g., IgG2a and IgG2b) of immunoglobulin molecule. Non-limiting examples of human constant regions are described in the art, *e.g.*, see Kabat E A et al., (1991) supra.

[000106] In some embodiments, agents binding to transferrin receptor, e.g., anti-TfR antibodies, are capable of targeting muscle cell and/or (e.g., and) mediate the transportation of an agent across the blood brain barrier. Transferrin receptors are internalizing cell surface receptors that transport transferrin across the cellular membrane and participate in the regulation and homeostasis of intracellular iron levels. Some aspects of the disclosure provide transferrin receptor binding proteins, which are capable of binding to transferrin receptor. Antibodies that bind, e.g. specifically bind, to a transferrin receptor may be internalized into the cell, e.g. through receptor-mediated endocytosis, upon binding to a transferrin receptor.

[000107] Provided herein, in some aspects, are humanized antibodies that bind to transferrin receptor with high specificity and affinity. In some embodiments, the humanized anti-TfR antibody described herein specifically binds to any extracellular epitope of a transferrin receptor or an epitope that becomes exposed to an antibody. In some embodiments, the humanized anti-TfR antibodies provided herein bind specifically to transferrin receptor from human, non-human primates, mouse, rat, *etc*. In some embodiments, the humanized anti-TfR antibodies provided herein bind to human transferrin receptor. In some embodiments, the humanized anti-TfR antibody described herein binds to an amino acid segment of a human or non-human primate transferrin receptor, as provided in SEQ ID NOs: 105-108. In some embodiments, the humanized anti-TfR antibody described herein binds to an amino acid segment corresponding to amino acids 90-96 of a human transferrin receptor as set forth in SEQ ID NO: 105, which is not in the apical domain of the transferrin receptor. In some embodiments, the humanized anti-TfR antibodies described herein binds to TfR1 but does not bind to TfR2.

[000108] In some embodiments, an anti-TFR antibody specifically binds a TfR1 (e.g., a human or non-human primate TfR1) with binding affinity (e.g., as indicated by Kd) of at least about 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, or less. In some embodiments, the anti-TfR antibodies described herein binds to TfR1 with a KD of sub-nanomolar range. In some embodiments, the anti-TfR antibodies described herein selectively binds to transferrin receptor 1 (TfR1) but do not bind to transferrin receptor 2 (TfR2). In some embodiments, the anti-TfR antibodies described herein binds to human TfR1 and cyno TfR1 (e.g., with a Kd of 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M,

or less), but does not bind to a mouse TfR1. The affinity and binding kinetics of the anti-TfR antibody can be tested using any suitable method including but not limited to biosensor technology (e.g., OCTET or BIACORE). In some embodiments, binding of any one of the anti-TfR antibody described herein does not complete with or inhibit transferrin binding to the TfR1. In some embodiments, binding of any one of the anti-TfR antibody described herein does not complete with or inhibit HFE-beta-2-microglobulin binding to the TfR1.

[000109] The anti-TfR antibodies described herein are humanized antibodies. The CDR and variable region amino acid sequences of the mouse monoclonal anti-TfR antibody from which the humanized anti-TfR antibodies described herein are derived are provided in Table 2.

Table 2. Mouse Monoclonal Anti-TfR Antibodies

Ab	No. system	IMGT	Kabat	Chothia
3-A4	CDR-H1	GFNIKDDY (SEQ ID NO: 1)	DDYMY (SEQ ID NO: 7)	GFNIKDD (SEQ ID NO: 12)
	CDR-H2	IDPENGDT (SEQ ID NO: 2)	WIDPENGDTEYASKFQD (SEQ ID NO: 8)	ENG (SEQ ID NO: 13)
	CDR-H3	TLWLRRGLDY (SEQ ID NO: 3)	WLRRGLDY (SEQ ID NO: 9)	LRRGLD (SEQ ID NO: 14)
	CDR-L1	KSLLHSNGYTY (SEQ ID NO: 4)	RSSKSLLHSNGYTYLF (SEQ ID NO: 10)	SKSLLHSNGYTY (SEQ ID NO: 15)
	CDR-L2	RMS (SEQ ID NO: 5)	RMSNLAS (SEQ ID NO: 11)	RMS (SEQ ID NO: 5)
	CDR-L3	MQHLEYPFT (SEQ ID NO: 6)	MQHLEYPFT (SEQ ID NO: 6)	HLEYPF (SEQ ID NO: 16)
	VH	EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYMYWVKQRPEQGLEWIGWIDPENGDT EYASKFQDKATVTADTSSNTAYLQLSSLTSEDTAVYYCTLWLRRGLDYWGQGTSVTVS S (SEQ ID NO: 17)		
	VL	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGYTYLFWFLQRPGQSPQLLIYRMSNLA SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGGGTKLEIK (SEQ ID NO: 18)		
3-A4 N54T*	CDR-H1	GFNIKDDY (SEQ ID NO: 1)	DDYMY (SEQ ID NO: 7)	GFNIKDD (SEQ ID NO: 12)
	CDR-H2	IDPETGDT (SEQ ID NO: 19)	WIDPETGDTEYASKFQD (SEQ ID NO: 20)	ETG (SEQ ID NO: 21)
	CDR-H3	TLWLRRGLDY (SEQ ID NO: 3)	WLRRGLDY (SEQ ID NO: 9)	LRRGLD (SEQ ID NO: 14)
	CDR-L1	KSLLHSNGYTY (SEQ ID NO: 4)	RSSKSLLHSNGYTYLF (SEQ ID NO: 10)	SKSLLHSNGYTY (SEQ ID NO: 15)
	CDR-L2	RMS (SEQ ID NO: 5)	RMSNLAS (SEQ ID NO: 11)	RMS(SEQ ID NO: 5)
	CDR-L3	MQHLEYPFT (SEQ ID NO: 6)	MQHLEYPFT (SEQ ID NO: 6)	HLEYPF (SEQ ID NO: 16)
	VH	EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYMYWVKQRPEQGLEWIGWIDPETGDT EYASKFQDKATVTADTSSNTAYLQLSSLTSEDTAVYYCTLWLRRGLDYWGQGTSVTVS S (SEQ ID NO: 22)		
	VL	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGYTYLFWFLQRPGQSPQLLIYRMSNLA SGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGGGTKLEIK (SEQ ID NO: 18)		
3-A4 N54S*	CDR-H1	GFNIKDDY (SEQ ID NO: 1)	DDYMY (SEQ ID NO: 7)	GFNIKDD (SEQ ID NO: 12)

	CDR-H2	IDPESGDT (SEQ ID NO: 23)	WIDPESGDTEYASKFQD (SEQ ID NO: 24)	ESG (SEQ ID NO: 25)
	CDR-H3	TLWLRRGLDY (SEQ ID NO: 3)	WLRRGLDY (SEQ ID NO: 9)	LRRGLD (SEQ ID NO: 14)
	CDR-L1	KSLLHSNGYTY (SEQ ID NO: 4)	RSSKSLLHSNGYTYLF (SEQ ID NO: 10)	SKSLLHSNGYTY (SEQ ID NO: 15)
	CDR-L2	RMS (SEQ ID NO: 5)	RMSNLAS (SEQ ID NO: 11)	RMS (SEQ ID NO: 5)
	CDR-L3	MQHLEYPFT (SEQ ID NO: 6)	MQHLEYPFT (SEQ ID NO: 6)	HLEYPF (SEQ ID NO: 16)
	VH	EVQLQQSGAELVRPGASVKLSCTASGFNIKDDYMYWVKQRPEQGLEWIGWIDPESGDTEYASKFQDKATVTADTSSNTAYLQLSSLTSEDTAVYYCTLWLRRGLDYWGQGTSVTVSS (SEQ ID NO: 26)		
	VL	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGYTYLFWFLQRPGQSPQLLIYRMSNLASGVPDRFSGSGSGTAFTLRISRVEAEDVGVYYCMQHLEYPFTFGGGTKLEIK (SEQ ID NO: 18)		
3-M12	CDR-H1	GYSITSGYY (SEQ ID NO: 27)	SGYYWN (SEQ ID NO: 33)	GYSITSGY (SEQ ID NO: 38)
	CDR-H2	ITFDGAN (SEQ ID NO: 28)	YITFDGANNYNPSLKN (SEQ ID NO: 34)	FDG (SEQ ID NO: 39)
	CDR-H3	TRSSYDYDVLDY (SEQ ID NO: 29)	SSYDYDVLDY (SEQ ID NO: 35)	SYDYDVLD (SEQ ID NO: 40)
	CDR-L1	QDISNF (SEQ ID NO: 30)	RASQDISNFLN (SEQ ID NO: 36)	SQDISNF (SEQ ID NO: 41)
	CDR-L2	YTS (SEQ ID NO: 31)	YTSRLHS (SEQ ID NO: 37)	YTS (SEQ ID NO: 31)
	CDR-L3	QQGHTLPYT (SEQ ID NO: 32)	QQGHTLPYT (SEQ ID NO: 32)	GHTLPY (SEQ ID NO: 42)
	VH	DVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITFDGANNYNPSLKNRISITRDTSKNQFFLKLTSVTTEDTATYYCTRSSYDYDVLDYWGQGTTLTVSS (SEQ ID NO: 43)		
	VL	DIQMTQTTSSLSASLGDRVTISCRASQDISNFLNWYQQRPDGTVKLLIYYTSRLHSGVPSRFSGSGSGTDFSLTVSNLEQEDIATYFCQQGHTLPYTFGGGTKLEIK (SEQ ID NO: 44)		
5-H12	CDR-H1	GYSFTDYC (SEQ ID NO: 45)	DYCIN (SEQ ID NO: 51)	GYSFTDY (SEQ ID NO: 56)
	CDR-H2	IYPGSGNT (SEQ ID NO: 46)	WIYPGSGNTRYSERFKG (SEQ ID NO: 52)	GSG (SEQ ID NO: 57)
	CDR-H3	AREDYYPYHGMDY (SEQ ID NO: 47)	EDYYPYHGMDY (SEQ ID NO: 53)	DYYPYHGMD (SEQ ID NO: 58)
	CDR-L1	ESVDGYDNSF (SEQ ID NO: 48)	RASESVDGYDNSFMH (SEQ ID NO: 54)	SESVDGYDNSF (SEQ ID NO: 59)
	CDR-L2	RAS (SEQ ID NO: 49)	RASNLES (SEQ ID NO: 55)	RAS (SEQ ID NO: 49)
	CDR-L3	QQSSEDPWT (SEQ ID NO: 50)	QQSSEDPWT (SEQ ID NO: 50)	SSEDPW (SEQ ID NO: 60)
	VH	QIQLQQSGPELVRPGASVKISCKASGYSFTDYCINWVNQRPGQGLEWIGWIYPGSGNTRYSERFKGKATLTVDTSSNTAYMQLSSLTSEDSAVYFCAREDYYPYHGMDYWGQGTSVTVSS (SEQ ID NO: 61)		
	VL	DIVLTQSPTSLAVSLGQRATISCRASESVDGYDNSFMHWYQQKPGQPPKLLIFRASNLESGIPARFSGSGSRTDFTLTINPVEAADVATYYCQQSSEDPWTFGGGTKLEIK (SEQ ID NO: 62)		
5-H12 C33Y*	CDR-H1	GYSFTDYY (SEQ ID NO: 63)	DYYIN (SEQ ID NO: 64)	GYSFTDY (SEQ ID NO: 56)
	CDR-H2	IYPGSGNT (SEQ ID NO: 46)	WIYPGSGNTRYSERFKG (SEQ ID NO: 52)	GSG (SEQ ID NO: 57)
	CDR-H3	AREDYYPYHGMDY (SEQ ID NO: 47)	EDYYPYHGMDY (SEQ ID NO: 53)	DYYPYHGMD (SEQ ID NO: 58)
	CDR-L1	ESVDGYDNSF (SEQ ID NO: 48)	RASESVDGYDNSFMH (SEQ ID NO: 54)	SESVDGYDNSF (SEQ ID NO: 59)

	CDR-L2	RAS (SEQ ID NO: 49)	RASNLES (SEQ ID NO: 55)	RAS (SEQ ID NO: 49)
	CDR-L3	QQSSEDPWT (SEQ ID NO: 50)	QQSSEDPWT (SEQ ID NO: 50)	SSEDPW (SEQ ID NO: 60)
	VH	QIQLQQSGPELVRPGASVKISCKASGYSFTDYYINWVNQRPGQGLEWIGWIYPGSGNTR YSERFKGKATLTVDTSSNTAYMQLSSLTSEDSAVYFCAREDYYPYHGMDYWGQGTSV TVSS (SEQ ID NO: 65)		
	VL	DIVLTQSPTSLAVSLGQRATISCRASESVDGYDNSFMHWYQQKPGQPPKLLIFRASNLES GIPARFSGSGSRTDFTLTINPVEAADVATYYCQQSSEDPWTFGGGTKLEIK (SEQ ID NO: 62)		
5-H12 C33D*	CDR-H1	GYSFTDYD (SEQ ID NO: 66)	DYDIN (SEQ ID NO: 67)	GYSFTDY (SEQ ID NO: 56)
	CDR-H2	IYPGSGNT (SEQ ID NO: 46)	WIYPGSGNTRYSERFKG (SEQ ID NO: 52)	GSG (SEQ ID NO: 57)
	CDR-H3	AREDYYPYHGMDY (SEQ ID NO: 47)	EDYYPYHGMDY (SEQ ID NO: 53)	DYYPYHGMD (SEQ ID NO: 58)
	CDR-L1	ESVDGYDNSF (SEQ ID NO: 48)	RASESVDGYDNSFMH (SEQ ID NO: 54)	SESVDGYDNSF (SEQ ID NO: 59)
	CDR-L2	RAS (SEQ ID NO: 49)	RASNLES (SEQ ID NO: 55)	RAS (SEQ ID NO: 49)
	CDR-L3	QQSSEDPWT (SEQ ID NO: 50)	QQSSEDPWT (SEQ ID NO: 50)	SSEDPW (SEQ ID NO: 60)
	VH	QIQLQQSGPELVRPGASVKISCKASGYSFTDYDINWVNQRPGQGLEWIGWIYPGSGNTRY SERFKGKATLTVDTSSNTAYMQLSSLTSEDSAVYFCAREDYYPYHGMDYWGQGTSVTV SS (SEQ ID NO: 68)		
	VL	DIVLTQSPTSLAVSLGQRATISCRASESVDGYDNSFMHWYQQKPGQPPKLLIFRASNLES GIPARFSGSGSRTDFTLTINPVEAADVATYYCQQSSEDPWTFGGGTKLEIK (SEQ ID NO: 62)		

** mutation positions are according to Kabat numbering of the respective VH sequences containing the mutations*

[000110] In some embodiments, the anti-TfR antibody of the present disclosure is a humanized variant of any one of the anti-TfR antibodies provided in Table 2. In some embodiments, the anti-TfR antibody of the present disclosure comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 that are the same as the CDR-H1, CDR-H2, and CDR-H3 in any one of the anti-TfR antibodies provided in Table 2, and comprises a humanized heavy chain variable region and/or (e.g., and) a humanized light chain variable region.

[000111] Humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some embodiments, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or

substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs derived from one or more CDRs from the original antibody. Humanized antibodies may also involve affinity maturation.

[000112] Humanized antibodies and methods of making them are known, e.g., as described in Almagro et al., Front. Biosci. 13:1619-1633 (2008); Riechmann et al., Nature 332:323-329 (1988); Queen et al., Proc. Nat'l Acad. Sci. USA 86:10029-10033 (1989); U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri et al., Methods 36:25-34 (2005); Padlan et al., Mol. Immunol. 28:489-498 (1991); Dall'Acqua et al., Methods 36:43-60 (2005); Osbourn et al., Methods 36:61-68 (2005); and Klimka et al., Br. J. Cancer, 83:252-260 (2000), the contents of all of which are incorporated herein by reference. Human framework regions that may be used for humanization are described in e.g., Sims et al. J. Immunol. 151:2296 (1993); Carter et al., Proc. Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immunol., 151:2623 (1993); Almagro et al., Front. Biosci. 13:1619-1633 (2008)); Baca et al., J. Biol. Chem. 272:10678-10684 (1997); and Rosok et al., J Biol. Chem. 271:22611-22618 (1996), the contents of all of which are incorporated herein by reference.

[000113] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising one or more amino acid variations (e.g., in the VH framework region) as compared with any one of the VHs listed in Table 2, and/or (e.g., and) a humanized VL comprising one or more amino acid variations (e.g., in the VL framework region) as compared with any one of the VLs listed in Table 2.

[000114] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH of any of the anti-TfR antibodies listed in Table 2 (e.g., any one of SEQ ID NOs: 17, 22, 26, 43, 61, 65, and 68). Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8,

7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL of any one of the anti-TfR antibodies listed in Table 2 (e.g., any one of SEQ ID NOs: 18, 44, and 62).

[000115] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH of any of the anti-TfR antibodies listed in Table 2 (e.g., any one of SEQ ID NOs: 17, 22, 26, 43, 61, 65, and 68). Alternatively or in addition (e.g., in addition), In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising an amino acid sequence that is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL of any of the anti-TfR antibodies listed in Table 2 (e.g., any one of SEQ ID NOs: 18, 44, and 62).

[000116] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 1 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 19, or SEQ ID NO: 23 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 3 (according to the IMGT definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH as set forth in SEQ ID NO: 17, SEQ ID NO: 22, or SEQ ID NO: 26. Alternatively or in addition (e.g., in addition), the anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 4 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 5 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the IMGT definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 18.

[000117] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 1 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 19, or SEQ ID NO: 23 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 3 (according to

the IMGT definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: 17, SEQ ID NO: 22, or SEQ ID NO: 26. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 4 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 5 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the IMGT definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in any one of SEQ ID NO: 18.

[000118] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 7 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 20, or SEQ ID NO: 24 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 9 (according to the Kabat definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH as set forth in SEQ ID NO: 17, SEQ ID NO: 22, or SEQ ID NO: 26. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 10 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 11 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the Kabat definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 18.

[000119] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 7 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 8, SEQ ID NO: 20, or SEQ ID NO: 24 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 9 (according to the Kabat definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: 17, SEQ ID

NO: 22, or SEQ ID NO: 26. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 10 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 11 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 6 (according to the Kabat definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in any one of SEQ ID NO: 18.

[000120] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 12 (according to the Chothia definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 21, or SEQ ID NO: 25 (according to the Chothia definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 14 (according to the Chothia definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH as set forth in SEQ ID NO: 17, SEQ ID NO: 22 or SEQ ID NO: 26. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 15 (according to the Chothia definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 5 (according to the Chothia definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 16 (according to the Chothia definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 18.

[000121] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 12 (according to the Chothia definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 21, or SEQ ID NO: 25 (according to the Chothia definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 14 (according to the Chothia definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: SEQ ID NO: 17, SEQ ID NO: 22 or SEQ ID NO: 26. Alternatively or in addition (e.g., in addition), the anti-TfR antibody of the present disclosure comprises a humanized VL

comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 15 (according to the Chothia definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 5 (according to the Chothia definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 16 (according to the Chothia definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in any one of SEQ ID NO: 18.

[000122] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 27 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 28 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 29 (according to the IMGT definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH as set forth in SEQ ID NO: 43. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 30 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 31 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 32 (according to the IMGT definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 44.

[000123] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 27 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 28 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 29 (according to the IMGT definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: 43. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 30 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 31 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 32 (according to the IMGT definition system), and is at least 75% (e.g., 75%,

80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in SEQ ID NO: 44.

[000124] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 33 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 34 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 35 (according to the Kabat definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH as set forth in SEQ ID NO: 43. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 36 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 37 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 32 (according to the Kabat definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 44.

[000125] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 33 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 34 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 35 (according to the Kabat definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: 43. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 36 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 37 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 32 (according to the Kabat definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in SEQ ID NO: 44.

[000126] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence

of SEQ ID NO: 38 (according to the Chothia definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 39 (according to the Chothia definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 40 (according to the Chothia definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH as set forth in SEQ ID NO: 43. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 41 (according to the Chothia definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 31 (according to the Chothia definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 42 (according to the Chothia definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 44.

[000127] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 38 (according to the Chothia definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 39 (according to the Chothia definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 40 (according to the Chothia definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: 43. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 41 (according to the Chothia definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 31 (according to the Chothia definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 42 (according to the Chothia definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in SEQ ID NO: 44.

[000128] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 45, SEQ ID NO: 63, or SEQ ID NO: 66 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 46 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 47 (according to the IMGT definition system), and containing no more than 25 amino acid

variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH as set forth in SEQ ID NO: 61, SEQ ID NO: 65, or SEQ ID NO: 68. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 48 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 49 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 50 (according to the IMGT definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 62.

[000129] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 45, SEQ ID NO: 63, or SEQ ID NO: 66 (according to the IMGT definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 46 (according to the IMGT definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 47 (according to the IMGT definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 68. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 48 (according to the IMGT definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 49 (according to the IMGT definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 50 (according to the IMGT definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in SEQ ID NO: 62.

[000130] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 51, SEQ ID NO: 64, or SEQ ID NO: 67 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 52 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 53 (according to the Kabat definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH

as set forth in SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 68. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 54 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 55 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 50 (according to the Kabat definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 62.

[000131] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 51, SEQ ID NO: 64, or SEQ ID NO: 67 (according to the Kabat definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 52 (according to the Kabat definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 53 (according to the Kabat definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 68. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 54 (according to the Kabat definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 55 (according to the Kabat definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 50 (according to the Kabat definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in SEQ ID NO: 62.

[000132] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 56 (according to the Chothia definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 57 (according to the Chothia definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 58 (according to the Chothia definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VH as set forth in SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 68. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1

having the amino acid sequence of SEQ ID NO: 59 (according to the Chothia definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 49 (according to the Chothia definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 60 (according to the Chothia definition system), and containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) in the framework regions as compared with the VL as set forth in SEQ ID NO: 62.

[000133] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising a CDR-H1 having the amino acid sequence of SEQ ID NO: 56 (according to the Chothia definition system), a CDR-H2 having the amino acid sequence of SEQ ID NO: 57 (according to the Chothia definition system), a CDR-H3 having the amino acid sequence of SEQ ID NO: 58 (according to the Chothia definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VH as set forth in SEQ ID NO: 61, SEQ ID NO: 65, SEQ ID NO: 68. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising a CDR-L1 having the amino acid sequence of SEQ ID NO: 59 (according to the Chothia definition system), a CDR-L2 having the amino acid sequence of SEQ ID NO: 49 (according to the Chothia definition system), and a CDR-L3 having the amino acid sequence of SEQ ID NO: 60 (according to the Chothia definition system), and is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical in the framework regions to the VL as set forth in SEQ ID NO: 62.

[000134] Examples of amino acid sequences of the humanized anti-TfR antibodies described herein are provided in Table 3.

Table 3. Variable Regions of Humanized Anti-TfR Antibodies

Antibody	Variable Region Amino Acid Sequence**
3A4 VH3 (N54T*)/Vκ4	V_H: EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WID PETGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGL DY**WGQGTLVTVSS (SEQ ID NO: 69)
	V_L: DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**R MSNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGT KVEIK (SEQ ID NO: 70)
3A4 VH3 (N54S*)/Vκ4	V_H: EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WID PESGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGL DY**WGQGTLVTVSS (SEQ ID NO: 71)

Antibody	Variable Region Amino Acid Sequence**
	V_L: DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**R MSNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGT KVEIK (SEQ ID NO: 70)
3A4 VH3 /Vκ4	VH: EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WID PENGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGL DY**WGQGTLVTVSS (SEQ ID NO: 72)
	VL: DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**R MSNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGT KVEIK (SEQ ID NO: 70)
3M12 VH3/Vκ2	VH: QVQLQESGPGLVKPSQTLSLTCSVTGYSIT**SGYYWN**WIRQPPGKGLEWMG**YIT FDGANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVL DY**WGQGTTVTVSS (SEQ ID NO: 73)
	VL: DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRL HS**GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC**QQGHTLPYT**FGQGTKLEIK (SEQ ID NO: 74)
3M12 VH3/Vκ3	VH: QVQLQESGPGLVKPSQTLSLTCSVTGYSIT**SGYYWN**WIRQPPGKGLEWMG**YIT FDGANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVL DY**WGQGTTVTVSS (SEQ ID NO: 73)
	VL: DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRL HS**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQGHTLPYT**FGQGTKLEIK (SEQ ID NO: 75)
3M12 VH4/Vκ2	VH: QVQLQESGPGLVKPSQTLSLTCTVTGYSIT**SGYYWN**WIRQPPGKGLEWIG**YITF DGANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLD Y**WGQGTTVTVSS (SEQ ID NO: 76)
	VL: DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRL HS**GVPSRFSGSGSGTDFTLTISSLQPEDFATYFC**QQGHTLPYT**FGQGTKLEIK (SEQ ID NO: 74)
3M12 VH4/Vκ3	VH: QVQLQESGPGLVKPSQTLSLTCTVTGYSIT**SGYYWN**WIRQPPGKGLEWIG**YITF DGANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLD Y**WGQGTTVTVSS (SEQ ID NO: 76)
	VL: DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRL HS**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQGHTLPYT**FGQGTKLEIK (SEQ ID NO: 75)
5H12 VH5 (C33Y*)/Vκ3	VH: QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYYIN**WVRQAPGQGLEWMG**WI YPGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPY HGMDY**WGQGTLVTVSS (SEQ ID NO: 77)
	VL: DIVLTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF**R ASNLES**GVPDRFSGSGSRTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGTK LEIK (SEQ ID NO: 78)
5H12 VH5 (C33D*)/Vκ4	VH: QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYDIN**WVRQAPGQGLEWMG**WI YPGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPY HGMDY**WGQGTLVTVSS (SEQ ID NO: 79)
	VL: DIVMTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF **RASNLES**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGT KLEIK (SEQ ID NO: 80)

Antibody	Variable Region Amino Acid Sequence**
5H12 VH5 (C33Y*)/Vκ4	VH: QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYYIN**WVRQAPGQGLEWMG**WI YPGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPY HGMDY**WGQGTLVTVSS (SEQ ID NO: 77)
	VL: DIVMTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF **RASNLES**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGT KLEIK (SEQ ID NO: 80)

** mutation positions are according to Kabat numbering of the respective VH sequences containing the mutations*
*** CDRs according to the Kabat numbering system are bolded*

[000135] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the CDR-H1, CDR-H2, and CDR-H3 of any one of the anti-TfR antibodies provided in Table 2 and comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) amino acid variations in the framework regions as compared with the respective humanized VH provided in Table 3. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a humanized VL comprising the CDR-L1, CDR-L2, and CDR-L3 of any one of the anti-TfR antibodies provided in Table 2 and comprises one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) amino acid variations in the framework regions as compared with the respective humanized VL provided in Table 3.

[000136] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 69, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 70. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 69 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 70.

[000137] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 71, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 70. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 71 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 70.

[000138] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 72, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 70. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 72 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 70.

[000139] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 73, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 74. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 73 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 74.

[000140] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 73, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 75. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 73 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 75.

[000141] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 76, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 74. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 76 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 74.

[000142] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80%

(e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 76, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 75. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 76 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 75.

[000143] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 77, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 78. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 77 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 78.

[000144] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 79, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 80. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 79 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 80.

[000145] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 77, and/or (e.g., and) a humanized VL comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 80. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a humanized VH comprising the amino acid sequence of SEQ ID NO: 77 and a humanized VL comprising the amino acid sequence of SEQ ID NO: 80.

[000146] In some embodiments, the humanized anti-TfR antibody described herein is a full-length IgG, which can include a heavy constant region and a light constant region from a human antibody. In some embodiments, the heavy chain of any of the anti-TfR antibodies as described herein may comprises a heavy chain constant region (CH) or a portion thereof (e.g.,

CH1, CH2, CH3, or a combination thereof). The heavy chain constant region can of any suitable origin, e.g., human, mouse, rat, or rabbit. In one specific example, the heavy chain constant region is from a human IgG (a gamma heavy chain), e.g., IgG1, IgG2, or IgG4. An example of a human IgG1 constant region is given below:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 81)

[000147] In some embodiments, the heavy chain of any of the anti-TfR antibodies described herein comprises a mutant human IgG1 constant region. For example, the introduction of LALA mutations (a mutant derived from mAb b12 that has been mutated to replace the lower hinge residues Leu234 Leu235 with Ala234 and Ala235) in the CH2 domain of human IgG1 is known to reduce Fcg receptor binding (Bruhns, P., et al . (2009) and Xu, D. et al. (2000)). The mutant human IgG1 constant region is provided below (mutations bonded and underlined):

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPE**A**
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 82)

[000148] In some embodiments, the light chain of any of the anti-TfR antibodies described herein may further comprise a light chain constant region (CL), which can be any CL known in the art. In some examples, the CL is a kappa light chain. In other examples, the CL is a lambda light chain. In some embodiments, the CL is a kappa light chain, the sequence of which is provided below:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 83)

[000149] Other antibody heavy and light chain constant regions are well known in the art, e.g., those provided in the IMGT database (www.imgt.org) or at www.vbase2.org/vbstat.php., both of which are incorporated by reference herein.

[000150] In some embodiments, the humanized anti-TfR antibody described herein comprises a heavy chain comprising any one of the VH as listed in Table 3 or any variants thereof and a heavy chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 81 or SEQ ID NO: 82. In some embodiments, the humanized anti-TfR antibody described herein comprises a heavy chain comprising any one of the VH as listed in Table 3 or any variants thereof and a heavy chain constant region that contains no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with SEQ ID NO: 81 or SEQ ID NO: 82. In some embodiments, the humanized anti-TfR antibody described herein comprises a heavy chain comprising any one of the VH as listed in Table 3 or any variants thereof and a heavy chain constant region as set forth in SEQ ID NO: 81. In some embodiments, the humanized anti-TfR antibody described herein comprises heavy chain comprising any one of the VH as listed in Table 3 or any variants thereof and a heavy chain constant region as set forth in SEQ ID NO: 82.

[000151] In some embodiments, the humanized anti-TfR antibody described herein comprises a light chain comprising any one of the VL as listed in Table 3 or any variants thereof and a light chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 83. In some embodiments, the humanized anti-TfR antibody described herein comprises a light chain comprising any one of the VL as listed in Table 3 or any variants thereof and a light chain constant region contains no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with SEQ ID NO: 83. In some embodiments, the humanized anti-TfR antibody described herein comprises a light chain comprising any one of the VL as listed in Table 3 or any variants thereof and a light chain constant region set forth in SEQ ID NO: 83.

[000152] Examples of IgG heavy chain and light chain amino acid sequences of the anti-TfR antibodies described are provided in Table 4 below.

Table 4. Heavy chain and light chain sequences of examples of humanized anti-TfR IgGs

Antibody	IgG Heavy Chain/Light Chain Sequences**
3A4 VH3 (N54T*)/Vκ4	Heavy Chain (with wild type human IgG1 constant region) EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WIDP** **ETGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGLD** **Y**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO: 84)
	Light Chain (with kappa light chain constant region) DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**RM** **SNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)
3A4 VH3 (N54S*)/Vκ4	Heavy Chain (with wild type human IgG1 constant region) EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WIDP** **ESGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGLDY** WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO: 86)
	Light Chain (with kappa light chain constant region) DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**RM** **SNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)
3A4 VH3 /Vκ4	Heavy Chain (with wild type human IgG1 constant region) EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WIDP** **ENGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGLD** **Y**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO: 87)
	Light Chain (with kappa light chain constant region) DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**RM** **SNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)
3M12 VH3/Vκ2	Heavy Chain (with wild type human IgG1 constant region) QVQLQESGPGLVKPSQTLSLTCSVTGYSIT**SGYYWN**WIRQPPGKGLEWMG**YITF** **DGANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLDY** WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO: 88)

Antibody	IgG Heavy Chain/Light Chain Sequences**
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRLH** **SGVPSRFSGSGSGTDFTLTISSLQPEDFATYFC<u>QQGHTLPYT</u>**FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 89)
3M12 VH3/Vκ3	Heavy Chain (with wild type human IgG1 constant region) QVQLQESGPGLVKPSQTLSLTCSVTGYSIT**SGYYWN**WIRQPPGKGLEWMG**YITF** **DGANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLDY** WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGK (SEQ ID NO: 88)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRLH** **SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC<u>QQGHTLPYT</u>**FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 90)
3M12 VH4/Vκ2	Heavy Chain (with wild type human IgG1 constant region) QVQLQESGPGLVKPSQTLSLTCTVTGYSIT**SGYYWN**WIRQPPGKGLEWIG**YITFD** **GANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLDYW** GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK (SEQ ID NO: 91)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRLH** **SGVPSRFSGSGSGTDFTLTISSLQPEDFATYFC<u>QQGHTLPYT</u>**FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 89)
3M12 VH4/Vκ3	Heavy Chain (with wild type human IgG1 constant region) QVQLQESGPGLVKPSQTLSLTCTVTGYSIT**SGYYWN**WIRQPPGKGLEWIG**YITFD** **GANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLDYW** GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH YTQKSLSLSPGK (SEQ ID NO: 91)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRLH** **SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC<u>QQGHTLPYT</u>**FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 90)

Antibody	IgG Heavy Chain/Light Chain Sequences**
5H12 VH5 (C33Y*)/Vκ3	Heavy Chain (with wild type human IgG1 constant region) QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYYIN**WVRQAPGQGLEWMG**WIY** **PGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPYH** **GMDY**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK (SEQ ID NO: 92)
	Light Chain (with kappa light chain constant region) DIVLTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF**RA** **SNLES**GVPDRFSGSGSRTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 93)
5H12 VH5 (C33D*)/Vκ4	Heavy Chain (with wild type human IgG1 constant region) QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYDIN**WVRQAPGQGLEWMG**WIY** **PGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPYH** **GMDY**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK (SEQ ID NO: 94)
	Light Chain (with kappa light chain constant region) DIVMTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF**R** **ASNLES**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGTKLE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 95)
5H12 VH5 (C33Y*)/Vκ4	Heavy Chain (with wild type human IgG1 constant region) QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYYIN**WVRQAPGQGLEWMG**WIY** **PGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPYH** **GMDY**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK (SEQ ID NO: 92)
	Light Chain (with kappa light chain constant region) DIVMTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF**R** **ASNLES**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGTKLE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 95)

** mutation positions are according to Kabat numbering of the respective VH sequences containing the mutations*
*** CDRs according to the Kabat numbering system are bolded; VH/VL sequences underlined*

[000153] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the heavy chain as set forth in any one of SEQ ID NOs: 84, 86, 87, 88, 91, 92, and 94. Alternatively or in addition (e.g., in addition), the

humanized anti-TfR antibody of the present disclosure comprises a light chain containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the light chain as set forth in any one of SEQ ID NOs: 85, 89, 90, 93, and 95.

[000154] In some embodiments, the humanized anti-TfR antibody described herein comprises a heavy chain comprising an amino acid sequence that is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical to any one of SEQ ID NOs: 84, 86, 87, 88, 91, 92, and 94. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody described herein comprises a light chain comprising an amino acid sequence that is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical to any one of SEQ ID NOs: 85, 89, 90, 93, and 95. In some embodiments, the anti-TfR antibody described herein comprises a heavy chain comprising the amino acid sequence of any one of SEQ ID NOs: 84, 86, 87, 88, 91, 92, and 94. Alternatively or in addition (e.g., in addition), the anti-TfR antibody described herein comprises a light chain comprising the amino acid sequence of any one of SEQ ID NOs: 85, 89, 90, 93, and 95.

[000155] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 84, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 85. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 84 and a light chain comprising the amino acid sequence of SEQ ID NO: 85.

[000156] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 86, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 85. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 86 and a light chain comprising the amino acid sequence of SEQ ID NO: 85.

[000157] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 87, and/or (e.g., and) a

light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 85. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 87 and a light chain comprising the amino acid sequence of SEQ ID NO: 85.

[000158] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 88, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 89. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 88 and a light chain comprising the amino acid sequence of SEQ ID NO: 89.

[000159] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 88, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 90. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 88 and a light chain comprising the amino acid sequence of SEQ ID NO: 90.

[000160] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 91, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 89. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 89.

[000161] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 91, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 90. In some embodiments, the humanized anti-TfR

antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of SEQ ID NO: 90.

[000162] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 92, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 93. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 92 and a light chain comprising the amino acid sequence of SEQ ID NO: 93.

[000163] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 94, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 95. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 94 and a light chain comprising the amino acid sequence of SEQ ID NO: 95.

[000164] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 92, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 95. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 92 and a light chain comprising the amino acid sequence of SEQ ID NO: 95.

[000165] In some embodiments, the anti-TfR antibody is a Fab fragment, Fab' fragment, or $F(ab')_2$ fragment of an intact antibody (full-length antibody). Antigen binding fragment of an intact antibody (full-length antibody) can be prepared via routine methods (e.g., recombinantly or by digesting the heavy chain constant region of a full length IgG using an enzyme such as papain). For example, $F(ab')_2$ fragments can be produced by pepsin or papain digestion of an antibody molecule, and Fab' fragments that can be generated by reducing the disulfide bridges of $F(ab')_2$ fragments. In some embodiments, a heavy chain constant region in

a Fab fragment of the anti-TfR1 antibody described herein comprises the amino acid sequence of:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 96)

[000166] In some embodiments, the humanized anti-TfR antibody described herein comprises a heavy chain comprising any one of the VH as listed in Table 3 or any variants thereof and a heavy chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 96. In some embodiments, the humanized anti-TfR antibody described herein comprises a heavy chain comprising any one of the VH as listed in Table 3 or any variants thereof and a heavy chain constant region that contains no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with SEQ ID NO: 96. In some embodiments, the humanized anti-TfR antibody described herein comprises a heavy chain comprising any one of the VH as listed in Table 3 or any variants thereof and a heavy chain constant region as set forth in SEQ ID NO: 96.

[000167] In some embodiments, the humanized anti-TfR antibody described herein comprises a light chain comprising any one of the VL as listed in Table 3 or any variants thereof and a light chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 83. In some embodiments, the humanized anti-TfR antibody described herein comprises a light chain comprising any one of the VL as listed in Table 3 or any variants thereof and a light chain constant region contains no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with SEQ ID NO: 83. In some embodiments, the humanized anti-TfR antibody described herein comprises a light chain comprising any one of the VL as listed in Table 3 or any variants thereof and a light chain constant region set forth in SEQ ID NO: 83.

[000168] Examples of Fab heavy chain and light chain amino acid sequences of the anti-TfR antibodies described are provided in Table 5 below.

Table 5. Heavy chain and light chain sequences of examples of humanized anti-TfR Fabs

Antibody	Fab Heavy Chain/Light Chain Sequences**
3A4 VH3 (N54T*)/Vκ4	Heavy Chain (with partial human IgG1 constant region) EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WIDP ETGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGLD** YWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHT (SEQ ID NO: 97)

Antibody	Fab Heavy Chain/Light Chain Sequences**
	Light Chain (with kappa light chain constant region) DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**RM** **SNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)
3A4 VH3 (N54S*)/Vκ4	Heavy Chain (with partial human IgG1 constant region) EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WIDP** **ESGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGLDY** WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHT (SEQ ID NO: 98)
	Light Chain (with kappa light chain constant region) DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**RM** **SNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)
3A4 VH3 /Vκ4	Heavy Chain (with partial human IgG1 constant region) EVQLVQSGSELKKPGASVKVSCTASGFNIK**DDYMY**WVRQPPGKGLEWIG**WIDP** **ENGDTEYASKFQD**RVTVTADTSTNTAYMELSSLRSEDTAVYYCTL**WLRRGLD** **Y**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNS GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHT (SEQ ID NO: 99)
	Light Chain (with kappa light chain constant region) DIVMTQSPLSLPVTPGEPASISC**RSSKSLLHSNGYTYLF**WFQQRPGQSPRLLIY**RM** **SNLAS**GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC**MQHLEYPFT**FGGGTKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)
3M12 VH3/Vκ2	Heavy Chain (with partial human IgG1 constant region) QVQLQESGPGLVKPSQTLSLTCSVTGYSIT**SGYYWN**WIRQPPGKGLEWMG**YITF** **DGANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLDY** WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHT (SEQ ID NO: 100)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRLH** **SGVPSRFSGSGSGTDFTLTISSLQPEDFATYFC**QQGHTLPYT**FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 89)
3M12 VH3/Vκ3	Heavy Chain (with partial human IgG1 constant region) QVQLQESGPGLVKPSQTLSLTCSVTGYSIT**SGYYWN**WIRQPPGKGLEWMG**YITF** **DGANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLDY** WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHT (SEQ ID NO: 100)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRLH** **SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQGHTLPYT**FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 90)
3M12 VH4/Vκ2	Heavy Chain (with partial human IgG1 constant region) QVQLQESGPGLVKPSQTLSLTCTVTGYSIT**SGYYWN**WIRQPPGKGLEWIG**YITFD** **GANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLDYW** GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHT (SEQ ID NO: 101)

Antibody	Fab Heavy Chain/Light Chain Sequences**
3M12 VH4/Vκ3	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRLH** **SG**VPSRFSGSGSGTDFTLTISSLQPEDFATYFC**QQGHTLPYT**FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 89)
	Heavy Chain (with partial human IgG1 constant region) QVQLQESGPGLVKPSQTLSLTCTVTGYSIT**SGYYWN**WIRQPPGKGLEWIG**YITFD** **GANNYNPSLKN**RVSISRDTSKNQFSLKLSSVTAEDTATYYCTR**SSYDYDVLDY**W GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHT (SEQ ID NO: 101)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC**RASQDISNFLN**WYQQKPGQPVKLLIY**YTSRLH** **SG**VPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQGHTLPYT**FGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 90)
5H12 VH5 (C33Y*)/Vκ3	Heavy Chain (with partial human IgG1 constant region) QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYYIN**WVRQAPGQGLEWMG**WIY** **PGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPYH** **GMDY**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHT (SEQ ID NO: 102)
	Light Chain (with kappa light chain constant region) DIVLTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF**RA** **SNLES**GVPDRFSGSGSRTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 93)
5H12 VH5 (C33D*)/Vκ4	Heavy Chain (with partial human IgG1 constant region) QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYDIN**WVRQAPGQGLEWMG**WIY** **PGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPYH** **GMDY**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHT (SEQ ID NO: 103)
	Light Chain (with kappa light chain constant region) DIVMTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF**R** **ASNLES**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGTKLE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 95)
5H12 VH5 (C33Y*)/Vκ4	Heavy Chain (with partial human IgG1 constant region) QVQLVQSGAEVKKPGASVKVSCKASGYSFT**DYYIN**WVRQAPGQGLEWMG**WIY** **PGSGNTRYSERFKG**RVTITRDTSASTAYMELSSLRSEDTAVYYCAR**EDYYPYH** **GMDY**WGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTV SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHT (SEQ ID NO: 102)
	Light Chain (with kappa light chain constant region) DIVMTQSPDSLAVSLGERATINC**RASESVDGYDNSFMH**WYQQKPGQPPKLLIF**R** **ASNLES**GVPDRFSGSGSGTDFTLTISSLQAEDVAVYYC**QQSSEDPWT**FGQGTKLE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 95)

** mutation positions are according to Kabat numbering of the respective VH sequences containing the mutations*

*** CDRs according to the Kabat numbering system are bolded; VH/VL sequences underlined*

[000169] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the heavy chain as set forth in any one of SEQ ID NOs: 97-103. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody of the present disclosure comprises a light chain containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the light chain as set forth in any one of SEQ ID NOs: 85, 89, 90, 93, and 95.

[000170] In some embodiments, the humanized anti-TfR antibody described herein comprises a heavy chain comprising an amino acid sequence that is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical to any one of SEQ ID NOs: 97-103. Alternatively or in addition (e.g., in addition), the humanized anti-TfR antibody described herein comprises a light chain comprising an amino acid sequence that is at least 75% (e.g., 75%, 80%, 85%, 90%, 95%, 98%, or 99%) identical to any one of SEQ ID NOs: 85, 89, 90, 93, and 95. In some embodiments, the anti-TfR antibody described herein comprises a heavy chain comprising the amino acid sequence of any one of SEQ ID NOs: 97-103. Alternatively or in addition (e.g., in addition), the anti-TfR antibody described herein comprises a light chain comprising the amino acid sequence of any one of SEQ ID NOs: 85, 89, 90, 93, and 95.

[000171] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 97, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 85. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 97 and a light chain comprising the amino acid sequence of SEQ ID NO: 85.

[000172] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 98, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 85. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid

sequence of SEQ ID NO: 98 and a light chain comprising the amino acid sequence of SEQ ID NO: 85.

[000173] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 99, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 85. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 99 and a light chain comprising the amino acid sequence of SEQ ID NO: 85.

[000174] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 100, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 89. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 100 and a light chain comprising the amino acid sequence of SEQ ID NO: 89.

[000175] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 100, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 90. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 100 and a light chain comprising the amino acid sequence of SEQ ID NO: 90.

[000176] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 101, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 89. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 101 and a light chain comprising the amino acid sequence of SEQ ID NO: 89.

[000177] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 101, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 90. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 101 and a light chain comprising the amino acid sequence of SEQ ID NO: 90.

[000178] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 102, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 93. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 and a light chain comprising the amino acid sequence of SEQ ID NO: 93.

[000179] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 103, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 95. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 103 and a light chain comprising the amino acid sequence of SEQ ID NO: 95.

[000180] In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) identical to SEQ ID NO: 102, and/or (e.g., and) a light chain comprising an amino acid sequence that is at least 80% identical (e.g., 80%, 85%, 90%, 95%, 98%, or 99%) to SEQ ID NO: 95. In some embodiments, the humanized anti-TfR antibody of the present disclosure comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 and a light chain comprising the amino acid sequence of SEQ ID NO: 95.

[000181] In some embodiments, the humanized anti-TfR receptor antibodies described herein can be in any antibody form, including, but not limited to, intact (i.e., full-length)

antibodies, antigen-binding fragments thereof (such as Fab, Fab', F(ab')2, Fv), single chain antibodies, bi-specific antibodies, or nanobodies. In some embodiments, humanized the anti-TfR antibody described herein is a scFv. In some embodiments, the humanized anti-TfR antibody described herein is a scFv-Fab (e.g., scFv fused to a portion of a constant region). In some embodiments, the anti-TfR receptor antibody described herein is a scFv fused to a constant region (e.g., human IgG1 constant region as set forth in SEQ ID NO: 81 or SEQ ID NO: 82, or a portion thereof such as the Fc portion) at either the N-terminus of C-terminus.

[000182] In some embodiments, conservative mutations can be introduced into antibody sequences (e.g., CDRs or framework sequences) at positions where the residues are not likely to be involved in interacting with a target antigen (e.g., transferrin receptor), for example, as determined based on a crystal structure. In some embodiments, one, two or more mutations (*e.g.,* amino acid substitutions) are introduced into the Fc region of an anti-TfR antibody described herein (*e.g.,* in a CH2 domain (residues 231-340 of human IgG1) and/or (e.g., and) CH3 domain (residues 341-447 of human IgG1) and/or (e.g., and) the hinge region, with numbering according to the Kabat numbering system (*e.g.,* the EU index in Kabat)) to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding and/or (e.g., and) antigen-dependent cellular cytotoxicity.

[000183] In some embodiments, one, two or more mutations (*e.g.,* amino acid substitutions) are introduced into the hinge region of the Fc region (CH1 domain) such that the number of cysteine residues in the hinge region are altered (*e.g.,* increased or decreased) as described in, e.g., U.S. Pat. No. 5,677,425. The number of cysteine residues in the hinge region of the CH1 domain can be altered to, *e.g.,* facilitate assembly of the light and heavy chains, or to alter (*e.g.,* increase or decrease) the stability of the antibody or to facilitate linker conjugation.

[000184] In some embodiments, one, two or more mutations (*e.g.,* amino acid substitutions) are introduced into the Fc region of a muscle-targeting antibody described herein (e.g., in a CH2 domain (residues 231-340 of human IgG1) and/or (e.g., and) CH3 domain (residues 341-447 of human IgG1) and/or (e.g., and) the hinge region, with numbering according to the Kabat numbering system (*e.g.,* the EU index in Kabat)) to increase or decrease the affinity of the antibody for an Fc receptor (*e.g.*, an activated Fc receptor) on the surface of an effector cell. Mutations in the Fc region of an antibody that decrease or increase the affinity of an antibody for an Fc receptor and techniques for introducing such mutations into the Fc receptor or fragment thereof are known to one of skill in the art. Examples of mutations in the Fc receptor of an antibody that can be made to alter the affinity of the antibody for an Fc

receptor are described in, e.g., Smith P et al., (2012) PNAS 109: 6181-6186, U.S. Pat. No. 6,737,056, and International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631, which are incorporated herein by reference.

[000185] In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to alter (*e.g.*, decrease or increase) half-life of the antibody *in vivo*. See, e.g., International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631; and U.S. Pat. Nos. 5,869,046, 6,121,022, 6,277,375 and 6,165,745 for examples of mutations that will alter (*e.g.*, decrease or increase) the half-life of an antibody *in vivo*.

[000186] In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to decrease the half-life of the anti-anti-TfR antibody *in vivo*. In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to increase the half-life of the antibody *in vivo*. In some embodiments, the antibodies can have one or more amino acid mutations (*e.g.*, substitutions) in the second constant (CH2) domain (residues 231-340 of human IgG1) and/or (e.g., and) the third constant (CH3) domain (residues 341-447 of human IgG1), with numbering according to the EU index in Kabat (Kabat E A et al., (1991) supra). In some embodiments, the constant region of the IgG1 of an antibody described herein comprises a methionine (M) to tyrosine (Y) substitution in position 252, a serine (S) to threonine (T) substitution in position 254, and a threonine (T) to glutamic acid (E) substitution in position 256, numbered according to the EU index as in Kabat. See U.S. Pat. No. 7,658,921, which is incorporated herein by reference. This type of mutant IgG, referred to as "YTE mutant" has been shown to display fourfold increased half-life as compared to wild-type versions of the same antibody (see Dall'Acqua W F et al., (2006) J Biol Chem 281: 23514-24). In some embodiments, an antibody comprises an IgG constant domain comprising one, two, three or more amino acid substitutions of amino acid residues at positions 251-257, 285-290, 308-314, 385-389, and 428-436, numbered according to the EU index as in Kabat.

[000187] In some embodiments, one, two or more amino acid substitutions are introduced into an IgG constant domain Fc region to alter the effector function(s) of the anti-anti-TfR antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Pat. Nos.

5,624,821 and 5,648,260. In some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain can reduce Fc receptor binding of the circulating antibody thereby increasing tumor localization. See, e.g., U.S. Pat. Nos. 5,585,097 and 8,591,886 for a description of mutations that delete or inactivate the constant domain and thereby increase tumor localization. In some embodiments, one or more amino acid substitutions may be introduced into the Fc region of an antibody described herein to remove potential glycosylation sites on Fc region, which may reduce Fc receptor binding (see, *e.g.,* Shields R L et al., (2001) J Biol Chem 276: 6591-604).

[000188] In some embodiments, one or more amino in the constant region of an anti-TfR antibody described herein can be replaced with a different amino acid residue such that the antibody has altered Clq binding and/or (e.g., and) reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Pat. No. 6,194,551 (Idusogie et al). In some embodiments, one or more amino acid residues in the N-terminal region of the CH2 domain of an antibody described herein are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in International Publication No. WO 94/29351. In some embodiments, the Fc region of an antibody described herein is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or (e.g., and) to increase the affinity of the antibody for an Fcγ receptor. This approach is described further in International Publication No. WO 00/42072.

[000189] In some embodiments, the heavy and/or (e.g., and) light chain variable domain(s) sequence(s) of the antibodies provided herein can be used to generate, for example, CDR-grafted, chimeric, humanized, or composite human antibodies or antigen-binding fragments, as described elsewhere herein. As understood by one of ordinary skill in the art, any variant, CDR-grafted, chimeric, humanized, or composite antibodies derived from any of the antibodies provided herein may be useful in the compositions and methods described herein and will maintain the ability to specifically bind transferrin receptor, such that the variant, CDR-grafted, chimeric, humanized, or composite antibody has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or more binding to transferrin receptor relative to the original antibody from which it is derived.

[000190] In some embodiments, the antibodies provided herein comprise mutations that confer desirable properties to the antibodies. For example, to avoid potential complications due to Fab-arm exchange, which is known to occur with native IgG4 mAbs, the antibodies provided herein may comprise a stabilizing 'Adair' mutation (Angal S., et al., "A single amino

acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody," Mol Immunol 30, 105-108; 1993), where serine 228 (EU numbering; residue 241 Kabat numbering) is converted to proline resulting in an IgG1-like hinge sequence. Accordingly, any of the antibodies may include a stabilizing 'Adair' mutation.

[000191] In some embodiments, an antibody is modified, e.g., modified via glycosylation, phosphorylation, sumoylation, and/or (e.g., and) methylation. In some embodiments, an antibody is a glycosylated antibody, which is conjugated to one or more sugar or carbohydrate molecules. In some embodiments, the one or more sugar or carbohydrate molecule are conjugated to the antibody via N-glycosylation, O-glycosylation, C-glycosylation, glypiation (GPI anchor attachment), and/or (e.g., and) phosphoglycosylation. In some embodiments, the one or more sugar or carbohydrate molecules are monosaccharides, disaccharides, oligosaccharides, or glycans. In some embodiments, the one or more sugar or carbohydrate molecule is a branched oligosaccharide or a branched glycan. In some embodiments, the one or more sugar or carbohydrate molecule includes a mannose unit, a glucose unit, an N-acetylglucosamine unit, an N-acetylgalactosamine unit, a galactose unit, a fucose unit, or a phospholipid unit. In some embodiments, there are about 1-10, about 1-5, about 5-10, about 1-4, about 1-3, or about 2 sugar molecules. In some embodiments, a glycosylated antibody is fully or partially glycosylated. In some embodiments, an antibody is glycosylated by chemical reactions or by enzymatic means. In some embodiments, an antibody is glycosylated in vitro or inside a cell, which may optionally be deficient in an enzyme in the N- or O- glycosylation pathway, e.g. a glycosyltransferase. In some embodiments, an antibody is functionalized with sugar or carbohydrate molecules as described in International Patent Application Publication WO2014065661, published on May 1, 2014, entitled, "*Modified antibody, antibody-conjugate and process for the preparation thereof*".

[000192] In some embodiments, any one of the anti-TfR1 antibodies described herein may comprise a signal peptide in the heavy and/or (e.g., and) light chain sequence (e.g., a N-terminal signal peptide). In some embodiments, the anti-TfR1 antibody described herein comprises any one of the VH and VL sequences, any one of the IgG heavy chain and light chain sequences, or any one of the Fab heavy chain and light chain sequences described herein, and further comprises a signal peptide (e.g., a N-terminal signal peptide). In some embodiments, the signal peptide comprises the amino acid sequence of MGWSCIILFLVATATGVHS (SEQ ID NO: 104).

Other known anti-transferrin receptor antibodies

[000193] Any other appropriate anti-transferrin receptor antibodies known in the art may be used as the muscle-targeting agent in the complexes disclosed herein. Examples of known anti-transferrin receptor antibodies, including associated references and binding epitopes, are listed in Table 8. In some embodiments, the anti-transferrin receptor antibody comprises the complementarity determining regions (CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3) of any of the anti-transferrin receptor antibodies provided herein, e.g., anti-transferrin receptor antibodies listed in Table 8.

[000194] Table 8 – List of anti-transferrin receptor antibody clones, including associated references and binding epitope information.

Antibody Clone Name	Reference(s)	Epitope / Notes
OKT9	US Patent. No. 4,364,934, filed 12/4/1979, entitled "MONOCLONAL ANTIBODY TO A HUMAN EARLY THYMOCYTE ANTIGEN AND METHODS FOR PREPARING SAME" Schneider C. et al. "Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9." J Biol Chem. 1982, 257:14, 8516-8522.	Apical domain of TfR (residues 305-366 of human TfR sequence XM_052730.3, available in GenBank)
(From JCR) Clone M11 Clone M23 Clone M27 Clone B84	• WO 2015/098989, filed 12/24/2014, "Novel anti-Transferrin receptor antibody that passes through blood-brain barrier" • US Patent No. 9,994,641, filed 12/24/2014, "Novel anti-Transferrin receptor antibody that passes through blood-brain barrier"	Apical domain (residues 230-244 and 326-347 of TfR) and protease-like domain (residues 461-473)
(From Genentech) 7A4, 8A2, 15D2, 10D11, 7B10, 15G11, 16G5, 13C3, 16G4, 16F6, 7G7, 4C2, 1B12, and 13D4	• WO 2016/081643, filed 5/26/2016, entitled "ANTI-TRANSFERRIN RECEPTOR ANTIBODIES AND METHODS OF USE" • US Patent No. 9,708,406, filed 5/20/2014, "Anti-transferrin receptor antibodies and methods of use"	Apical domain and non-apical regions
(From Armagen) 8D3	• Lee et al. "Targeting Rat Anti-Mouse Transferrin Receptor Monoclonal Antibodies through Blood-Brain	

	Barrier in Mouse" 2000, J Pharmacol. Exp. Ther., 292: 1048-1052. • US Patent App. 2010/077498, filed 9/11/2008, entitled "COMPOSITIONS AND METHODS FOR BLOOD-BRAIN BARRIER DELIVERY IN THE MOUSE"	
OX26	• Haobam, B. et al. 2014. Rab17-mediated recycling endosomes contribute to autophagosome formation in response to Group A Streptococcus invasion. Cellular microbiology. 16: 1806-21.	
DF1513	• Ortiz-Zapater E et al. Trafficking of the human transferrin receptor in plant cells: effects of tyrphostin A23 and brefeldin A. Plant J 48:757-70 (2006).	
1A1B2, 66IG10, MEM-189, JF0956, 29806, 1A1B2, TFRC/1818, 1E6, 66Ig10, TFRC/1059, Q1/71, 23D10, 13E4, TFRC/1149, ER-MP21, YTA74.4, BU54, 2B6, RI7 217	• Commercially available anti-transferrin receptor antibodies.	Novus Biologicals 8100 Southpark Way, A-8 Littleton CO 80120
(From INSERM) BA120g	• US Patent App. 2011/0311544A1, filed 6/15/2005, entitled "ANTI-CD71 MONOCLONAL ANTIBODIES AND USES THEREOF FOR TREATING MALIGNANT TUMOR CELLS"	Does not compete with OKT9
LUCA31	• US Patent No. 7,572,895, filed 6/7/2004, entitled "TRANSFERRIN RECEPTOR ANTIBODIES"	"LUCA31 epitope"
(Salk Institute) B3/25 T58/30	• Trowbridge, I.S. et al. "Anti-transferrin receptor monoclonal antibody and toxin–antibody conjugates affect growth of human tumour cells." Nature, 1981, volume 294, pages 171–173	
R17 217.1.3, 5E9C11,	• Commercially available anti-transferrin receptor antibodies.	BioXcell

OKT9 (BE0023 clone)		10 Technology Dr., Suite 2B West Lebanon, NH 03784-1671 USA
BK19.9, B3/25, T56/14 and T58/1	• Gatter, K.C. et al. "Transferrin receptors in human tissues: their distribution and possible clinical relevance." J Clin Pathol. 1983 May;36(5):539-45.	

Anti-TfR antibody
CDRH1 (SEQ ID NO: 2265)
CDRH2 (SEQ ID NO: 2266)
CDRH3 (SEQ ID NO: 2267)
CDRL1 (SEQ ID NO: 2268)
CDRL2 (SEQ ID NO: 2269)
CDRL3 (SEQ ID NO: 2270)
VH (SEQ ID NO: 2271)
VL (SEQ ID NO: 2272)

Additional Anti-TfR SEQ ID NOs

	VH/VL	CDR1	CDR2	CDR3
VH1	2280	2273	2274	2267
VH2	2281	2273	2275	2267
VH3	2282	2273	2276	2267
VH4	2283	2273	2275	2267
VL1	2284	2268	2269	115
VL2	2285	2268	2269	115
VL3	2286	2268	2277	2270
VL4	2287	2278	2279	2270

[000195] In some embodiments, transferrin receptor antibodies of the present disclosure include one or more of the CDR-H (*e.g.*, CDR-H1, CDR-H2, and CDR-H3) amino acid sequences from any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, transferrin receptor antibodies include the CDR-H1, CDR-H2, and CDR-H3 as provided for any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, anti-transferrin receptor antibodies include the CDR-L1, CDR-L2, and CDR-L3 as provided for any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, anti-transferrin antibodies include the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 as provided for any one of the anti-transferrin receptor antibodies selected from Table 8. The disclosure also includes any nucleic acid sequence that encodes a molecule comprising a CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, or CDR-L3 as provided for any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, antibody heavy and light chain CDR3 domains may play a particularly important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, anti-transferrin receptor antibodies of the disclosure may include at least the heavy and/or (e.g., and) light chain CDR3s of any one of the anti-transferrin receptor antibodies selected from Table 8.

[000196] In some examples, any of the anti- transferrin receptor antibodies of the disclosure have one or more CDR (*e.g.*, CDR-H or CDR-L) sequences substantially similar to any of the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and/or (e.g., and) CDR-L3

sequences from one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, the position of one or more CDRs along the VH (*e.g.*, CDR-H1, CDR-H2, or CDR-H3) and/or (e.g., and) VL (*e.g.*, CDR-L1, CDR-L2, or CDR-L3) region of an antibody described herein can vary by one, two, three, four, five, or six amino acid positions so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% of the binding of the original antibody from which it is derived). For example, in some embodiments, the position defining a CDR of any antibody described herein can vary by shifting the N-terminal and/or (e.g., and) C-terminal boundary of the CDR by one, two, three, four, five, or six amino acids, relative to the CDR position of any one of the antibodies described herein, so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% of the binding of the original antibody from which it is derived). In another embodiment, the length of one or more CDRs along the VH (*e.g.*, CDR-H1, CDR-H2, or CDR-H3) and/or (e.g., and) VL (*e.g.*, CDR-L1, CDR-L2, or CDR-L3) region of an antibody described herein can vary (*e.g.*, be shorter or longer) by one, two, three, four, five, or more amino acids, so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% of the binding of the original antibody from which it is derived).

[000197] Accordingly, in some embodiments, a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or (e.g., and) CDR-H3 described herein may be one, two, three, four, five or more amino acids shorter than one or more of the CDRs described herein (*e.g.*, CDRS from any of the anti-transferrin receptor antibodies selected from Table 8) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or (e.g., and) CDR-H3 described herein may be one, two, three, four, five or more amino acids longer than one or more of the CDRs described herein (*e.g.*, CDRS from any of the anti-transferrin receptor antibodies selected from Table 8) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at

least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, the amino portion of a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or (e.g., and) CDR-H3 described herein can be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, CDRS from any of the anti-transferrin receptor antibodies selected from Table 8) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, the carboxy portion of a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or (e.g., and) CDR-H3 described herein can be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, CDRS from any of the anti-transferrin receptor antibodies selected from Table 8) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (e.g., substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, the amino portion of a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or (e.g., and) CDR-H3 described herein can be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, CDRS from any of the anti-transferrin receptor antibodies selected from Table 8) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, the carboxy portion of a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or (e.g., and) CDR-H3 described herein can be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, CDRS from any of the anti-transferrin receptor antibodies selected from Table 8) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). Any method can be used to ascertain whether immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained, for example, using binding assays and conditions described in the art.

[000198] In some examples, any of the anti-transferrin receptor antibodies of the disclosure have one or more CDR (*e.g.*, CDR-H or CDR-L) sequences substantially similar to

any one of the anti-transferrin receptor antibodies selected from Table 8. For example, the antibodies may include one or more CDR sequence(s) from any of the anti-transferrin receptor antibodies selected from Table 8 containing up to 5, 4, 3, 2, or 1 amino acid residue variations as compared to the corresponding CDR region in any one of the CDRs provided herein (*e.g.*, CDRs from any of the anti-transferrin receptor antibodies selected from Table 8) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, any of the amino acid variations in any of the CDRs provided herein may be conservative variations. Conservative variations can be introduced into the CDRs at positions where the residues are not likely to be involved in interacting with a transferrin receptor protein (*e.g.*, a human transferrin receptor protein), for example, as determined based on a crystal structure. Some aspects of the disclosure provide transferrin receptor antibodies that comprise one or more of the heavy chain variable (VH) and/or (e.g., and) light chain variable (VL) domains provided herein. In some embodiments, any of the VH domains provided herein include one or more of the CDR-H sequences (*e.g.*, CDR-H1, CDR-H2, and CDR-H3) provided herein, for example, any of the CDR-H sequences provided in any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, any of the VL domains provided herein include one or more of the CDR-L sequences (*e.g.*, CDR-L1, CDR-L2, and CDR-L3) provided herein, for example, any of the CDR-L sequences provided in any one of the anti-transferrin receptor antibodies selected from Table 8.

[000199] In some embodiments, anti-transferrin receptor antibodies of the disclosure include any antibody that includes a heavy chain variable domain and/or (e.g., and) a light chain variable domain of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, anti-transferrin receptor antibodies of the disclosure include any antibody that includes the heavy chain variable and light chain variable pairs of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8.

[000200] Aspects of the disclosure provide anti-transferrin receptor antibodies having a heavy chain variable (VH) and/or (e.g., and) a light chain variable (VL) domain amino acid sequence homologous to any of those described herein. In some embodiments, the anti-transferrin receptor antibody comprises a heavy chain variable sequence or a light chain variable sequence that is at least 75% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the heavy chain variable sequence and/ or any light chain variable sequence of any anti-

transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, the homologous heavy chain variable and/or (e.g., and) a light chain variable amino acid sequences do not vary within any of the CDR sequences provided herein. For example, in some embodiments, the degree of sequence variation (*e.g.*, 75%, 80%, 85%, 90%, 95%, 98%, or 99%) may occur within a heavy chain variable and/or (e.g., and) a light chain variable sequence excluding any of the CDR sequences provided herein. In some embodiments, any of the anti-transferrin receptor antibodies provided herein comprise a heavy chain variable sequence and a light chain variable sequence that comprises a framework sequence that is at least 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the framework sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8.

[000201] In some embodiments, an anti-transferrin receptor antibody, which specifically binds to transferrin receptor (*e.g.*, human transferrin receptor), comprises a light chain variable VL domain comprising any of the CDR-L domains (CDR-L1, CDR-L2, and CDR-L3), or CDR-L domain variants provided herein, of any of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, an anti-transferrin receptor antibody, which specifically binds to transferrin receptor (*e.g.*, human transferrin receptor), comprises a light chain variable VL domain comprising the CDR-L1, the CDR-L2, and the CDR-L3 of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, the anti-transferrin receptor antibody comprises a light chain variable (VL) region sequence comprising one, two, three or four of the framework regions of the light chain variable region sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, the anti-transferrin receptor antibody comprises one, two, three or four of the framework regions of a light chain variable region sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to one, two, three or four of the framework regions of the light chain variable region sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, the light chain variable framework region that is derived from said amino acid sequence consists of said amino acid sequence but for the presence of up to 10 amino acid substitutions, deletions, and/or (e.g., and) insertions, preferably up to 10 amino acid substitutions. In some embodiments, the light chain variable framework region that is derived from said amino acid sequence consists of said amino acid sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid

residues being substituted for an amino acid found in an analogous position in a corresponding non-human, primate, or human light chain variable framework region.

[000202] In some embodiments, an anti-transferrin receptor antibody that specifically binds to transferrin receptor comprises the CDR-L1, the CDR-L2, and the CDR-L3 of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, the antibody further comprises one, two, three or all four VL framework regions derived from the VL of a human or primate antibody. The primate or human light chain framework region of the antibody selected for use with the light chain CDR sequences described herein, can have, for example, at least 70% (*e.g.*, at least 75%, 80%, 85%, 90%, 95%, 98%, or at least 99%) identity with a light chain framework region of a non-human parent antibody. The primate or human antibody selected can have the same or substantially the same number of amino acids in its light chain complementarity determining regions to that of the light chain complementarity determining regions of any of the antibodies provided herein, *e.g.*, any of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, the primate or human light chain framework region amino acid residues are from a natural primate or human antibody light chain framework region having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, at least 99% (or more) identity with the light chain framework regions of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8. In some embodiments, an anti-transferrin receptor antibody further comprises one, two, three or all four VL framework regions derived from a human light chain variable kappa subfamily. In some embodiments, an anti-transferrin receptor antibody further comprises one, two, three or all four VL framework regions derived from a human light chain variable lambda subfamily.

[000203] In some embodiments, any of the anti-transferrin receptor antibodies provided herein comprise a light chain variable domain that further comprises a light chain constant region. In some embodiments, the light chain constant region is a kappa, or a lambda light chain constant region. In some embodiments, the kappa or lambda light chain constant region is from a mammal, *e.g.,* from a human, monkey, rat, or mouse. In some embodiments, the light chain constant region is a human kappa light chain constant region. In some embodiments, the light chain constant region is a human lambda light chain constant region. It should be appreciated that any of the light chain constant regions provided herein may be variants of any of the light chain constant regions provided herein. In some embodiments, the light chain constant region comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%,

98%, or 99% identical to any of the light chain constant regions of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8.

[000204] In some embodiments, the anti-transferrin receptor antibody is any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8.

[000205] In some embodiments, an anti-transferrin receptor antibody comprises a VL domain comprising the amino acid sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 8, and wherein the constant regions comprise the amino acid sequences of the constant regions of an IgG, IgE, IgM, IgD, IgA or IgY immunoglobulin molecule, or a human IgG, IgE, IgM, IgD, IgA or IgY immunoglobulin molecule. In some embodiments, an anti-transferrin receptor antibody comprises any of the VL domains, or VL domain variants, and any of the VH domains, or VH domain variants, wherein the VL and VH domains, or variants thereof, are from the same antibody clone, and wherein the constant regions comprise the amino acid sequences of the constant regions of an IgG, IgE, IgM, IgD, IgA or IgY immunoglobulin molecule, any class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or any subclass (e.g., IgG2a and IgG2b) of immunoglobulin molecule. Non-limiting examples of human constant regions are described in the art, *e.g.*, see Kabat E A et al., (1991) supra.

[000206] In some embodiments, the muscle-targeting agent is a transferrin receptor antibody (e.g., the antibody and variants thereof as described in International Application Publication WO 2016/081643, incorporated herein by reference).

[000207] The heavy chain and light chain CDRs of the antibody according to different definition systems are provided in Table 9. The different definition systems, e.g., the Kabat definition, the Chothia definition, and/or (e.g., and) the contact definition have been described. See, e.g., (e.g., Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia et al., (1989) Nature 342:877; Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, Al-lazikani et al (1997) J. Molec. Biol. 273:927-948; and Almagro, J. Mol. Recognit. 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs).

Table 9 Heavy chain and light chain CDRs of a mouse transferrin receptor antibody

CDRs	Kabat	Chothia	Contact
CDR-H1	SYWMH (SEQ ID NO: 110)	GYTFTSY (SEQ ID NO: 116)	TSYWMH (SEQ ID NO: 118)
CDR-H2	EINPTNGRTNYIEKFKS (SEQ ID NO: 111)	NPTNGR (SEQ ID NO: 117)	WIGEINPTNGRTN (SEQ ID NO: 119)

CDR-H3	GTRAYHY (SEQ ID NO: 112)	GTRAYHY (SEQ ID NO: 112)	ARGTRA (SEQ ID NO: 120)
CDR-L1	RASDNLYSNLA (SEQ ID NO: 113)	RASDNLYSNLA (SEQ ID NO: 113)	YSNLAWY (SEQ ID NO: 121)
CDR-L2	DATNLAD (SEQ ID NO: 114)	DATNLAD (SEQ ID NO: 114)	LLVYDATNLA (SEQ ID NO: 122)
CDR-L3	QHFWGTPLT (SEQ ID NO: 115)	QHFWGTPLT (SEQ ID NO: 115)	QHFWGTPL (SEQ ID NO: 123)

[000208] The heavy chain variable domain (VH) and light chain variable domain sequences are also provided:

[000209] VH

QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGEINPTNG RTNYIEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARGTRAYHYWGQGTSVT VSS (SEQ ID NO: 124)

[000210] VL

DIQMTQSPASLSVSVGETVTITCRASDNLYSNLAWYQQKQGKSPQLLVYDATNLADG VPSRFSGSGSGTQYSLKINSLQSEDFGTYYCQHFWGTPLTFGAGTKLELK (SEQ ID NO: 125)

[000211] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, and a CDR-H3 that are the same as the CDR-H1, CDR-H2, and CDR-H3 shown in Table 9. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises a CDR-L1, a CDR-L2, and a CDR-L3 that are the same as the CDR-L1, CDR-L2, and CDR-L3 shown in Table 9.

[000212] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, and a CDR-H3, which collectively contains no more than 5 amino acid variations (e.g., no more than 5, 4, 3, 2, or 1 amino acid variation) as compared with the CDR-H1, CDR-H2, and CDR-H3 as shown in Table 9. "Collectively" means that the total number of amino acid variations in all of the three heavy chain CDRs is within the defined range. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure may comprise a CDR-L1, a CDR-L2, and a CDR-L3, which collectively contains no more than 5 amino acid variations (e.g., no more than 5, 4, 3, 2 or 1 amino acid variation) as compared with the CDR-L1, CDR-L2, and CDR-L3 as shown in Table 9.

[000213] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, and a CDR-H3, at least one of which contains no more than 3 amino acid variations (e.g., no more than 3, 2, or 1 amino acid variation) as compared with the counterpart heavy chain CDR as shown in Table 9. Alternatively or in

addition (e.g., in addition), the transferrin receptor antibody of the present disclosure may comprise CDR-L1, a CDR-L2, and a CDR-L3, at least one of which contains no more than 3 amino acid variations (e.g., no more than 3, 2, or 1 amino acid variation) as compared with the counterpart light chain CDR as shown in Table 9.

[000214] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-L3, which contains no more than 3 amino acid variations (e.g., no more than 3, 2, or 1 amino acid variation) as compared with the CDR-L3 as shown in Table 9. In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-L3 containing one amino acid variation as compared with the CDR-L3 as shown in Table 9. In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-L3 of QHFAGTPLT (SEQ ID NO: 126) according to the Kabat and Chothia definition system) or QHFAGTPL (SEQ ID NO: 127) according to the Contact definition system). In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1 and a CDR-L2 that are the same as the CDR-H1, CDR-H2, and CDR-H3 shown in Table 9, and comprises a CDR-L3 of QHFAGTPLT (SEQ ID NO: 126) according to the Kabat and Chothia definition system) or QHFAGTPL (SEQ ID NO: 127) according to the Contact definition system).

[000215] In some embodiments, the transferrin receptor antibody of the present disclosure comprises heavy chain CDRs that collectively are at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the heavy chain CDRs as shown in Table 9. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises light chain CDRs that collectively are at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the light chain CDRs as shown in Table 9.

[000216] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH comprising the amino acid sequence of SEQ ID NO: 124. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises a VL comprising the amino acid sequence of SEQ ID NO: 125.

[000217] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VH as set forth in SEQ ID NO: 124. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises a VL containing no more than 15 amino acid variations (e.g., no more than 20, 19,

18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VL as set forth in SEQ ID NO: 125.

[000218] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the VH as set forth in SEQ ID NO: 124. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises a VL comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the VL as set forth in SEQ ID NO: 125.

[000219] In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized antibody (e.g., a humanized variant of an antibody). In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 that are the same as the CDR-H1, CDR-H2, and CDR-H3 shown in Table 9, and comprises a humanized heavy chain variable region and/or (e.g., and) a humanized light chain variable region.

[000220] Humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some embodiments, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs derived from one or more CDRs from the original antibody. Humanized antibodies may also involve affinity maturation.

[000221] In some embodiments, humanization is achieved by grafting the CDRs (e.g., as shown in Table 9) into the IGKV1-NL1*01 and IGHV1-3*01 human variable domains. In

some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising one or more amino acid substitutions at positions 9, 13, 17, 18, 40, 45, and 70 as compared with the VL as set forth in SEQ ID NO: 125, and/or (e.g., and) one or more amino acid substitutions at positions 1, 5, 7, 11, 12, 20, 38, 40, 44, 66, 75, 81, 83, 87, and 108 as compared with the VH as set forth in SEQ ID NO: 124. In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising amino acid substitutions at all of positions 9, 13, 17, 18, 40, 45, and 70 as compared with the VL as set forth in SEQ ID NO: 125, and/or (e.g., and) amino acid substitutions at all of positions 1, 5, 7, 11, 12, 20, 38, 40, 44, 66, 75, 81, 83, 87, and 108 as compared with the VH as set forth in SEQ ID NO: 124.

[000222] In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized antibody and contains the residues at positions 43 and 48 of the VL as set forth in SEQ ID NO: 125. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure is a humanized antibody and contains the residues at positions 48, 67, 69, 71, and 73 of the VH as set forth in SEQ ID NO: 124.

[000223] The VH and VL amino acid sequences of an example humanized antibody that may be used in accordance with the present disclosure are provided:

[000224] Humanized VH

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQRLEWIGEINPTNG RTNYIEKFKSRATLTVDKSASTAYMELSSLRSEDTAVYYCARGTRAYHYWGQGTMV TVSS (SEQ ID NO: 128)

[000225] Humanized VL

DIQMTQSPSSLSASVGDRVTITCRASDNLYSNLAWYQQKPGKSPKLLVYDATNLADG VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQHFWGTPLTFGQGTKVEIK (SEQ ID NO: 129)

[000226] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH comprising the amino acid sequence of SEQ ID NO: 128. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises a VL comprising the amino acid sequence of SEQ ID NO: 129.

[000227] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VH as set forth in SEQ ID NO: 128. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure

comprises a VL containing no more than 15 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VL as set forth in SEQ ID NO: 129.

[000228] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the VH as set forth in SEQ ID NO: 128. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises a VL comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the VL as set forth in SEQ ID NO: 129.

[000229] In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising amino acid substitutions at one or more of positions 43 and 48 as compared with the VL as set forth in SEQ ID NO: 125, and/or (e.g., and) amino acid substitutions at one or more of positions 48, 67, 69, 71, and 73 as compared with the VH as set forth in SEQ ID NO: 124. In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising a S43A and/or (e.g., and) a V48L mutation as compared with the VL as set forth in SEQ ID NO: 125, and/or (e.g., and) one or more of A67V, L69I, V71R, and K73T mutations as compared with the VH as set forth in SEQ ID NO: 124.

[000230] In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising amino acid substitutions at one or more of positions 9, 13, 17, 18, 40, 43, 48, 45, and 70 as compared with the VL as set forth in SEQ ID NO: 125, and/or (e.g., and) amino acid substitutions at one or more of positions 1, 5, 7, 11, 12, 20, 38, 40, 44, 48, 66, 67, 69, 71, 73, 75, 81, 83, 87, and 108 as compared with the VH as set forth in SEQ ID NO: 124.

[000231] In some embodiments, the transferrin receptor antibody of the present disclosure is a chimeric antibody, which can include a heavy constant region and a light constant region from a human antibody. Chimeric antibodies refer to antibodies having a variable region or part of variable region from a first species and a constant region from a second species. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals (e.g., a non-human mammal such as mouse, rabbit, and rat), while the constant portions are homologous to the sequences in antibodies derived from another mammal such as human. In some embodiments, amino acid modifications can be made in the variable region and/or (e.g., and) the constant region.

[000232] In some embodiments, the transferrin receptor antibody described herein is a chimeric antibody, which can include a heavy constant region and a light constant region from a human antibody. Chimeric antibodies refer to antibodies having a variable region or part of variable region from a first species and a constant region from a second species. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals (*e.g.*, a non-human mammal such as mouse, rabbit, and rat), while the constant portions are homologous to the sequences in antibodies derived from another mammal such as human. In some embodiments, amino acid modifications can be made in the variable region and/or (e.g., and) the constant region.

[000233] In some embodiments, the heavy chain of any of the transferrin receptor antibodies as described herein may comprises a heavy chain constant region (CH) or a portion thereof (e.g., CH1, CH2, CH3, or a combination thereof). The heavy chain constant region can of any suitable origin, e.g., human, mouse, rat, or rabbit. In one specific example, the heavy chain constant region is from a human IgG (a gamma heavy chain), e.g., IgG1, IgG2, or IgG4. An example of a human IgG1 constant region is given below:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 130)

[000234] In some embodiments, the light chain of any of the transferrin receptor antibodies described herein may further comprise a light chain constant region (CL), which can be any CL known in the art. In some examples, the CL is a kappa light chain. In other examples, the CL is a lambda light chain. In some embodiments, the CL is a kappa light chain, the sequence of which is provided below:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 83)

[000235] Other antibody heavy and light chain constant regions are well known in the art, e.g., those provided in the IMGT database (www.imgt.org) or at www.vbase2.org/vbstat.php., both of which are incorporated by reference herein.

[000236] Examples of heavy chain and light chain amino acid sequences of the transferrin receptor antibodies described are provided below:

[000237] Heavy Chain (VH + human IgG1 constant region)

QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGEINPTNG
RTNYIEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARGTRAYHYWGQGTSVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 132)

[000238] Light Chain (VL + kappa light chain)

DIQMTQSPASLSVSVGETVTITCRASDNLYSNLAWYQQKQGKSPQLLVYDATNLADG
VPSRFSGSGSGTQYSLKINSLQSEDFGTYYCQHFWGTPLTFGAGTKLELKRTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 133)

[000239] Heavy Chain (humanized VH + human IgG1 constant region)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQRLEWIGEINPTNG
RTNYIEKFKSRATLTVDKSASTAYMELSSLRSEDTAVYYCARGTRAYHYWGQGTMV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 134)

[000240] Light Chain (humanized VL + kappa light chain)

DIQMTQSPSSLSASVGDRVTITCRASDNLYSNLAWYQQKPGKSPKLLVYDATNLADG
VPSRFSGSGSGTDYTLTISSLQPEDFATYYCQHFWGTPLTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 135)

[000241] In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 132. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody described herein comprises a light chain comprising

an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 133. In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 132. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody described herein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 133.

[000242] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a heavy chain containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the heavy chain as set forth in SEQ ID NO: 132. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises a light chain containing no more than 15 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the light chain as set forth in SEQ ID NO: 133.

[000243] In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 134. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody described herein comprises a light chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 135. In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 134. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody described herein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 135.

[000244] In some embodiments, the transferrin receptor antibody of the present disclosure comprises a heavy chain containing no more than 25 amino acid variations (e.g., no more than 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the heavy chain of humanized antibody as set forth in SEQ ID NO: 134. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody of the present disclosure comprises a light chain containing no more than 15 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the light chain of humanized antibody as set forth in SEQ ID NO: 135.

[000245] In some embodiments, the transferrin receptor antibody is an antigen binding fragment (Fab) of an intact antibody (full-length antibody). Antigen binding fragment of an intact antibody (full-length antibody) can be prepared via routine methods. For example,

F(ab')2 fragments can be produced by pepsin digestion of an antibody molecule, and Fab' fragments that can be generated by reducing the disulfide bridges of F(ab')2 fragments. Examples of Fab amino acid sequences of the transferrin receptor antibodies described herein are provided below:

[000246] Heavy Chain Fab (VH + a portion of human IgG1 constant region)
QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGEINPTNG
RTNYIEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARGTRAYHYWGQGTSVT
VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP
(SEQ ID NO: 136)

[000247] Heavy Chain Fab (humanized VH + a portion of human IgG1 constant region)
EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQRLEWIGEINPTNG
RTNYIEKFKSRATLTVDKSASTAYMELSSLRSEDTAVYYCARGTRAYHYWGQGTMV
TVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP (SEQ
ID NO: 137)

[000248] In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 136. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody described herein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 133.

[000249] In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 137. Alternatively or in addition (e.g., in addition), the transferrin receptor antibody described herein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 135.

[000250] The transferrin receptor antibodies described herein can be in any antibody form, including, but not limited to, intact (i.e., full-length) antibodies, antigen-binding fragments thereof (such as Fab, Fab', F(ab')2, Fv), single chain antibodies, bi-specific antibodies, or nanobodies. In some embodiments, the transferrin receptor antibody described herein is a scFv. In some embodiments, the transferrin receptor antibody described herein is a scFv-Fab (e.g., scFv fused to a portion of a constant region). In some embodiments, the transferrin receptor antibody described herein is a scFv fused to a constant region (e.g., human IgG1 constant region as set forth in SEQ ID NO: 130).

[000251] In some embodiments, any one of the anti-TfR antibodies described herein is produced by recombinant DNA technology in Chinese hamster ovary (CHO) cell suspension

culture, optionally in CHO-K1 cell (e.g., CHO-K1 cells derived from European Collection of Animal Cell Culture, Cat. No. 85051005) suspension culture.

[000252] In some embodiments, an antibody provided herein may have one or more post-translational modifications. In some embodiments, N-terminal cyclization, also called pyroglutamate formation (pyro-Glu), may occur in the antibody at N-terminal Glutamate (Glu) and/or Glutamine (Gln) residues during production. As such, it should be appreciated that an antibody specified as having a sequence comprising an N-terminal glutamate or glutamine residue encompasses antibodies that have undergone pyroglutamate formation resulting from a post-translational modification. In some embodiments, pyroglutamate formation occurs in a heavy chain sequence. In some embodiments, pyroglutamate formation occurs in a light chain sequence.

b. Other Muscle-Targeting Antibodies

[000253] In some embodiments, the muscle-targeting antibody is an antibody that specifically binds hemojuvelin, caveolin-3, Duchenne muscular dystrophy peptide, myosin Iib, or CD63. In some embodiments, the muscle-targeting antibody is an antibody that specifically binds a myogenic precursor protein. Exemplary myogenic precursor proteins include, without limitation, ABCG2, M-Cadherin/Cadherin-15, Caveolin-1, CD34, FoxK1, Integrin alpha 7, Integrin alpha 7 beta 1, MYF-5, MyoD, Myogenin, NCAM-1/CD56, Pax3, Pax7, and Pax9. In some embodiments, the muscle-targeting antibody is an antibody that specifically binds a skeletal muscle protein. Exemplary skeletal muscle proteins include, without limitation, alpha-Sarcoglycan, beta-Sarcoglycan, Calpain Inhibitors, Creatine Kinase MM/CKMM, eIF5A, Enolase 2/Neuron-specific Enolase, epsilon-Sarcoglycan, FABP3/H-FABP, GDF-8/Myostatin, GDF-11/GDF-8, Integrin alpha 7, Integrin alpha 7 beta 1, Integrin beta 1/CD29, MCAM/CD146, MyoD, Myogenin, Myosin Light Chain Kinase Inhibitors, NCAM-1/CD56, and Troponin I. In some embodiments, the muscle-targeting antibody is an antibody that specifically binds a smooth muscle protein. Exemplary smooth muscle proteins include, without limitation, alpha-Smooth Muscle Actin, VE-Cadherin, Caldesmon/CALD1, Calponin 1, Desmin, Histamine H2 R, Motilin R/GPR38, Transgelin/TAGLN, and Vimentin. However, it should be appreciated that antibodies to additional targets are within the scope of this disclosure and the exemplary lists of targets provided herein are not meant to be limiting.

c. Antibody Features/Alterations

[000254] In some embodiments, conservative mutations can be introduced into antibody sequences (e.g., CDRs or framework sequences) at positions where the residues are not likely

to be involved in interacting with a target antigen (e.g., transferrin receptor), for example, as determined based on a crystal structure. In some embodiments, one, two or more mutations (*e.g.*, amino acid substitutions) are introduced into the Fc region of a muscle-targeting antibody described herein (*e.g.*, in a CH2 domain (residues 231-340 of human IgG1) and/or (e.g., and) CH3 domain (residues 341-447 of human IgG1) and/or (e.g., and) the hinge region, with numbering according to the Kabat numbering system (*e.g.*, the EU index in Kabat)) to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding and/or (e.g., and) antigen-dependent cellular cytotoxicity.

[000255] In some embodiments, one, two or more mutations (*e.g.*, amino acid substitutions) are introduced into the hinge region of the Fc region (CH1 domain) such that the number of cysteine residues in the hinge region are altered (*e.g.*, increased or decreased) as described in, e.g., U.S. Pat. No. 5,677,425. The number of cysteine residues in the hinge region of the CH1 domain can be altered to, *e.g.*, facilitate assembly of the light and heavy chains, or to alter (*e.g.*, increase or decrease) the stability of the antibody or to facilitate linker conjugation.

[000256] In some embodiments, one, two or more mutations (*e.g.*, amino acid substitutions) are introduced into the Fc region of a muscle-targeting antibody described herein (e.g., in a CH2 domain (residues 231-340 of human IgG1) and/or (e.g., and) CH3 domain (residues 341-447 of human IgG1) and/or (c.g., and) the hinge region, with numbering according to the Kabat numbering system (*e.g.*, the EU index in Kabat)) to increase or decrease the affinity of the antibody for an Fc receptor (*e.g.*, an activated Fc receptor) on the surface of an effector cell. Mutations in the Fc region of an antibody that decrease or increase the affinity of an antibody for an Fc receptor and techniques for introducing such mutations into the Fc receptor or fragment thereof are known to one of skill in the art. Examples of mutations in the Fc receptor of an antibody that can be made to alter the affinity of the antibody for an Fc receptor are described in, e.g., Smith P et al., (2012) PNAS 109: 6181-6186, U.S. Pat. No. 6,737,056, and International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631, which are incorporated herein by reference.

[000257] In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to alter (*e.g.*, decrease or increase) half-life of the antibody *in vivo*. See, e.g., International Publication Nos. WO 02/060919; WO 98/23289; and WO 97/34631; and U.S. Pat. Nos. 5,869,046, 6,121,022,

6,277,375 and 6,165,745 for examples of mutations that will alter (*e.g.*, decrease or increase) the half-life of an antibody *in vivo*.

[000258] In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to decrease the half-life of the anti-transferrin receptor antibody *in vivo*. In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to increase the half-life of the antibody *in vivo*. In some embodiments, the antibodies can have one or more amino acid mutations (*e.g.*, substitutions) in the second constant (CH2) domain (residues 231-340 of human IgG1) and/or (e.g., and) the third constant (CH3) domain (residues 341-447 of human IgG1), with numbering according to the EU index in Kabat (Kabat E A et al., (1991) supra). In some embodiments, the constant region of the IgG1 of an antibody described herein comprises a methionine (M) to tyrosine (Y) substitution in position 252, a serine (S) to threonine (T) substitution in position 254, and a threonine (T) to glutamic acid (E) substitution in position 256, numbered according to the EU index as in Kabat. See U.S. Pat. No. 7,658,921, which is incorporated herein by reference. This type of mutant IgG, referred to as "YTE mutant" has been shown to display fourfold increased half-life as compared to wild-type versions of the same antibody (see Dall'Acqua W F et al., (2006) J Biol Chem 281: 23514-24). In some embodiments, an antibody comprises an IgG constant domain comprising one, two, three or more amino acid substitutions of amino acid residues at positions 251-257, 285-290, 308-314, 385-389, and 428-436, numbered according to the EU index as in Kabat.

[000259] In some embodiments, one, two or more amino acid substitutions are introduced into an IgG constant domain Fc region to alter the effector function(s) of the anti-transferrin receptor antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Pat. Nos. 5,624,821 and 5,648,260. In some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain can reduce Fc receptor binding of the circulating antibody thereby increasing tumor localization. See, e.g., U.S. Pat. Nos. 5,585,097 and 8,591,886 for a description of mutations that delete or inactivate the constant domain and thereby increase tumor localization. In some embodiments, one or more amino acid substitutions may be introduced into the Fc region of an antibody described herein to remove potential glycosylation sites on Fc region, which may reduce Fc receptor binding (see, *e.g.*, Shields R L et al., (2001) J Biol Chem 276: 6591-604).

[000260] In some embodiments, one or more amino in the constant region of a muscle-targeting antibody described herein can be replaced with a different amino acid residue such that the antibody has altered Clq binding and/or (e.g., and) reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in further detail in U.S. Pat. No. 6,194,551 (Idusogie et al). In some embodiments, one or more amino acid residues in the N-terminal region of the CH2 domain of an antibody described herein are altered to thereby alter the ability of the antibody to fix complement. This approach is described further in International Publication No. WO 94/29351. In some embodiments, the Fc region of an antibody described herein is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or (e.g., and) to increase the affinity of the antibody for an Fcγ receptor. This approach is described further in International Publication No. WO 00/42072.

[000261] In some embodiments, the heavy and/or (e.g., and) light chain variable domain(s) sequence(s) of the antibodies provided herein can be used to generate, for example, CDR-grafted, chimeric, humanized, or composite human antibodies or antigen-binding fragments, as described elsewhere herein. As understood by one of ordinary skill in the art, any variant, CDR-grafted, chimeric, humanized, or composite antibodies derived from any of the antibodies provided herein may be useful in the compositions and methods described herein and will maintain the ability to specifically bind transferrin receptor, such that the variant, CDR-grafted, chimeric, humanized, or composite antibody has at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% or more binding to transferrin receptor relative to the original antibody from which it is derived.

[000262] In some embodiments, the antibodies provided herein comprise mutations that confer desirable properties to the antibodies. For example, to avoid potential complications due to Fab-arm exchange, which is known to occur with native IgG4 mAbs, the antibodies provided herein may comprise a stabilizing 'Adair' mutation (Angal S., et al., "A single amino acid substitution abolishes the heterogeneity of chimeric mouse/human (IgG4) antibody," Mol Immunol 30, 105-108; 1993), where serine 228 (EU numbering; residue 241 Kabat numbering) is converted to proline resulting in an IgG1-like hinge sequence. Accordingly, any of the antibodies may include a stabilizing 'Adair' mutation.

[000263] As provided herein, antibodies of this disclosure may optionally comprise constant regions or parts thereof. For example, a VL domain may be attached at its C-terminal end to a light chain constant domain like Cκ or Cλ. Similarly, a VH domain or portion thereof may be attached to all or part of a heavy chain like IgA, IgD, IgE, IgG, and IgM, and any

isotype subclass. Antibodies may include suitable constant regions (see, for example, Kabat et al., Sequences of Proteins of Immunological Interest, No. 91-3242, National Institutes of Health Publications, Bethesda, Md. (1991)). Therefore, antibodies within the scope of this may disclosure include VH and VL domains, or an antigen binding portion thereof, combined with any suitable constant regions.

ii. Muscle-Targeting Peptides

[000264] Some aspects of the disclosure provide muscle-targeting peptides as muscle-targeting agents. Short peptide sequences (e.g., peptide sequences of 5-20 amino acids in length) that bind to specific cell types have been described. For example, cell-targeting peptides have been described in Vines e., et al., A. "Cell-penetrating and cell-targeting peptides in drug delivery" *Biochim Biophys Acta* 2008, 1786: 126-38; Jarver P., et al., "In vivo biodistribution and efficacy of peptide mediated delivery" *Trends Pharmacol Sci* 2010; 31: 528-35; Samoylova T.I., et al., "Elucidation of muscle-binding peptides by phage display screening" *Muscle Nerve* 1999; 22: 460-6; U.S. Patent No. 6,329,501, issued on December 11, 2001, entitled "METHODS AND COMPOSITIONS FOR TARGETING COMPOUNDS TO MUSCLE"; and Samoylov A.M., et al., "Recognition of cell-specific binding of phage display derived peptides using an acoustic wave sensor." *Biomol Eng* 2002; 18: 269-72; the entire contents of each of which are incorporated herein by reference. By designing peptides to interact with specific cell surface antigens (*e.g.*, receptors), selectivity for a desired tissue, e.g., muscle, can be achieved. Skeletal muscle-targeting has been investigated and a range of molecular payloads are able to be delivered. These approaches may have high selectivity for muscle tissue without many of the practical disadvantages of a large antibody or viral particle. Accordingly, in some embodiments, the muscle-targeting agent is a muscle-targeting peptide that is from 4 to 50 amino acids in length. In some embodiments, the muscle-targeting peptide is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length. Muscle-targeting peptides can be generated using any of several methods, such as phage display.

[000265] In some embodiments, a muscle-targeting peptide may bind to an internalizing cell surface receptor that is overexpressed or relatively highly expressed in muscle cells, e.g. a transferrin receptor, compared with certain other cells. In some embodiments, a muscle-targeting peptide may target, e.g., bind to, a transferrin receptor. In some embodiments, a peptide that targets a transferrin receptor may comprise a segment of a naturally occurring ligand, e.g., transferrin. In some embodiments, a peptide that targets a transferrin receptor is as

described in US Patent No. 6,743,893, filed 11/30/2000, "RECEPTOR-MEDIATED UPTAKE OF PEPTIDES THAT BIND THE HUMAN TRANSFERRIN RECEPTOR". In some embodiments, a peptide that targets a transferrin receptor is as described in Kawamoto, M. et al, "A novel transferrin receptor-targeted hybrid peptide disintegrates cancer cell membrane to induce rapid killing of cancer cells." BMC Cancer. 2011 Aug 18;11:359. In some embodiments, a peptide that targets a transferrin receptor is as described in US Patent No. 8,399,653, filed 5/20/2011, "TRANSFERRIN/TRANSFERRIN RECEPTOR-MEDIATED SIRNA DELIVERY".

[000266] As discussed above, examples of muscle targeting peptides have been reported. For example, muscle-specific peptides were identified using phage display library presenting surface heptapeptides. As one example a peptide having the amino acid sequence ASSLNIA (SEQ ID NO: 138) bound to C2C12 murine myotubes *in vitro*, and bound to mouse muscle tissue *in vivo*. Accordingly, in some embodiments, the muscle-targeting agent comprises the amino acid sequence ASSLNIA (SEQ ID NO: 138). This peptide displayed improved specificity for binding to heart and skeletal muscle tissue after intravenous injection in mice with reduced binding to liver, kidney, and brain. Additional muscle-specific peptides have been identified using phage display. For example, a 12 amino acid peptide was identified by phage display library for muscle targeting in the context of treatment for DMD. See, Yoshida D., et al., "Targeting of salicylate to skin and muscle following topical injections in rats." *Int J Pharm* 2002; 231: 177-84; the entire contents of which are hereby incorporated by reference. Here, a 12 amino acid peptide having the sequence SKTFNTHPQSTP (SEQ ID NO: 139) was identified and this muscle-targeting peptide showed improved binding to C2C12 cells relative to the ASSLNIA (SEQ ID NO: 138) peptide.

[000267] An additional method for identifying peptides selective for muscle (*e.g.*, skeletal muscle) over other cell types includes *in vitro* selection, which has been described in Ghosh D., et al., "Selection of muscle-binding peptides from context-specific peptide-presenting phage libraries for adenoviral vector targeting" *J Virol* 2005; 79: 13667-72; the entire contents of which are incorporated herein by reference. By pre-incubating a random 12-mer peptide phage display library with a mixture of non-muscle cell types, non-specific cell binders were selected out. Following rounds of selection the 12 amino acid peptide TARGEHKEEELI (SEQ ID NO: 140) appeared most frequently. Accordingly, in some embodiments, the muscle-targeting agent comprises the amino acid sequence TARGEHKEEELI (SEQ ID NO: 140).

[000268] A muscle-targeting agent may an amino acid-containing molecule or peptide. A muscle-targeting peptide may correspond to a sequence of a protein that preferentially binds to

a protein receptor found in muscle cells. In some embodiments, a muscle-targeting peptide contains a high propensity of hydrophobic amino acids, e.g. valine, such that the peptide preferentially targets muscle cells. In some embodiments, a muscle-targeting peptide has not been previously characterized or disclosed. These peptides may be conceived of, produced, synthesized, and/or (e.g., and) derivatized using any of several methodologies, e.g. phage displayed peptide libraries, one-bead one-compound peptide libraries, or positional scanning synthetic peptide combinatorial libraries. Exemplary methodologies have been characterized in the art and are incorporated by reference (Gray, B.P. and Brown, K.C. "Combinatorial Peptide Libraries: Mining for Cell-Binding Peptides" Chem Rev. 2014, 114:2, 1020–1081.; Samoylova, T.I. and Smith, B.F. "Elucidation of muscle-binding peptides by phage display screening." Muscle Nerve, 1999, 22:4. 460-6.). In some embodiments, a muscle-targeting peptide has been previously disclosed (see, e.g. Writer M.J. et al. "Targeted gene delivery to human airway epithelial cells with synthetic vectors incorporating novel targeting peptides selected by phage display." J. Drug Targeting. 2004;12:185; Cai, D. "BDNF-mediated enhancement of inflammation and injury in the aging heart." Physiol Genomics. 2006, 24:3, 191-7.; Zhang, L. "Molecular profiling of heart endothelial cells." Circulation, 2005, 112:11, 1601-11.; McGuire, M.J. et al. "In vitro selection of a peptide with high selectivity for cardiomyocytes in vivo." J Mol Biol. 2004, 342:1, 171-82.). Exemplary muscle-targeting peptides comprise an amino acid sequence of the following group: CQAQGQLVC (SEQ ID NO: 141), CSERSMNFC (SEQ ID NO: 142), CPKTRRVPC (SEQ ID NO: 143), WLSEAGPVVTVRALRGTGSW (SEQ ID NO: 144), ASSLNIA (SEQ ID NO: 138), CMQHSMRVC (SEQ ID NO: 145), and DDTRHWG (SEQ ID NO: 146). In some embodiments, a muscle-targeting peptide may comprise about 2-25 amino acids, about 2-20 amino acids, about 2-15 amino acids, about 2-10 amino acids, or about 2-5 amino acids. Muscle-targeting peptides may comprise naturally-occurring amino acids, e.g. cysteine, alanine, or non-naturally-occurring or modified amino acids. Non-naturally occurring amino acids include β-amino acids, homo-amino acids, proline derivatives, 3-substituted alanine derivatives, linear core amino acids, N-methyl amino acids, and others known in the art. In some embodiments, a muscle-targeting peptide may be linear; in other embodiments, a muscle-targeting peptide may be cyclic, e.g. bicyclic (see, e.g. Silvana, M.G. et al. Mol. Therapy, 2018, 26:1, 132–147.).

iii. Muscle-Targeting Receptor Ligands

[000269] A muscle-targeting agent may be a ligand, e.g. a ligand that binds to a receptor protein. A muscle-targeting ligand may be a protein, e.g. transferrin, which binds to an internalizing cell surface receptor expressed by a muscle cell. Accordingly, in some embodiments, the muscle-targeting agent is transferrin, or a derivative thereof that binds to a transferrin receptor. A muscle-targeting ligand may alternatively be a small molecule, e.g. a lipophilic small molecule that preferentially targets muscle cells relative to other cell types. Exemplary lipophilic small molecules that may target muscle cells include compounds comprising cholesterol, cholesteryl, stearic acid, palmitic acid, oleic acid, oleyl, linolene, linoleic acid, myristic acid, sterols, dihydrotestosterone, testosterone derivatives, glycerine, alkyl chains, trityl groups, and alkoxy acids.

iv. Muscle-Targeting Aptamers

[000270] A muscle-targeting agent may be an aptamer, e.g. an RNA aptamer, which preferentially targets muscle cells relative to other cell types. In some embodiments, a muscle-targeting aptamer has not been previously characterized or disclosed. These aptamers may be conceived of, produced, synthesized, and/or (e.g., and) derivatized using any of several methodologies, e.g. Systematic Evolution of Ligands by Exponential Enrichment. Exemplary methodologies have been characterized in the art and are incorporated by reference (Yan, A.C. and Levy, M. "Aptamers and aptamer targeted delivery" RNA biology, 2009, 6:3, 316-20.; Germer, K. et al. "RNA aptamers and their therapeutic and diagnostic applications." Int. J. Biochem. Mol. Biol. 2013; 4: 27–40.). In some embodiments, a muscle-targeting aptamer has been previously disclosed (see, e.g. Phillippou, S. et al. "Selection and Identification of Skeletal-Muscle-Targeted RNA Aptamers." Mol Ther Nucleic Acids. 2018, 10:199-214.; Thiel, W.H. et al. "Smooth Muscle Cell-targeted RNA Aptamer Inhibits Neointimal Formation." Mol Ther. 2016, 24:4, 779-87.). Exemplary muscle-targeting aptamers include the A01B RNA aptamer and RNA Apt 14. In some embodiments, an aptamer is a nucleic acid-based aptamer, an oligonucleotide aptamer or a peptide aptamer. In some embodiments, an aptamer may be about 5-15 kDa, about 5-10 kDa, about 10-15 kDa, about 1-5 Da, about 1-3 kDa, or smaller.

v. Other Muscle-Targeting Agents

[000271] One strategy for targeting a muscle cell (e.g., a skeletal muscle cell) is to use a substrate of a muscle transporter protein, such as a transporter protein expressed on the sarcolemma. In some embodiments, the muscle-targeting agent is a substrate of an influx transporter that is specific to muscle tissue. In some embodiments, the influx transporter is specific to skeletal muscle tissue. Two main classes of transporters are expressed on the

skeletal muscle sarcolemma, (1) the adenosine triphosphate (ATP) binding cassette (ABC) superfamily, which facilitate efflux from skeletal muscle tissue and (2) the solute carrier (SLC) superfamily, which can facilitate the influx of substrates into skeletal muscle. In some embodiments, the muscle-targeting agent is a substrate that binds to an ABC superfamily or an SLC superfamily of transporters. In some embodiments, the substrate that binds to the ABC or SLC superfamily of transporters is a naturally-occurring substrate. In some embodiments, the substrate that binds to the ABC or SLC superfamily of transporters is a non-naturally occurring substrate, for example, a synthetic derivative thereof that binds to the ABC or SLC superfamily of transporters.

[000272] In some embodiments, the muscle-targeting agent is a substrate of an SLC superfamily of transporters. SLC transporters are either equilibrative or use proton or sodium ion gradients created across the membrane to drive transport of substrates. Exemplary SLC transporters that have high skeletal muscle expression include, without limitation, the SATT transporter (ASCT1; SLC1A4), GLUT4 transporter (SLC2A4), GLUT7 transporter (GLUT7; SLC2A7), ATRC2 transporter (CAT-2; SLC7A2), LAT3 transporter (KIAA0245; SLC7A6), PHT1 transporter (PTR4; SLC15A4), OATP-J transporter (OATP5A1; SLC21A15), OCT3 transporter (EMT; SLC22A3), OCTN2 transporter (FLJ46769; SLC22A5), ENT transporters (ENT1; SLC29A1 and ENT2; SLC29A2), PAT2 transporter (SLC36A2), and SAT2 transporter (KIAA1382; SLC38A2). These transporters can facilitate the influx of substrates into skeletal muscle, providing opportunities for muscle targeting.

[000273] In some embodiments, the muscle-targeting agent is a substrate of an equilibrative nucleoside transporter 2 (ENT2) transporter. Relative to other transporters, ENT2 has one of the highest mRNA expressions in skeletal muscle. While human ENT2 (hENT2) is expressed in most body organs such as brain, heart, placenta, thymus, pancreas, prostate, and kidney, it is especially abundant in skeletal muscle. Human ENT2 facilitates the uptake of its substrates depending on their concentration gradient. ENT2 plays a role in maintaining nucleoside homeostasis by transporting a wide range of purine and pyrimidine nucleobases. The hENT2 transporter has a low affinity for all nucleosides (adenosine, guanosine, uridine, thymidine, and cytidine) except for inosine. Accordingly, in some embodiments, the muscle-targeting agent is an ENT2 substrate. Exemplary ENT2 substrates include, without limitation, inosine, 2′,3′-dideoxyinosine, and calofarabine. In some embodiments, any of the muscle-targeting agents provided herein are associated with a molecular payload (e.g., oligonucleotide payload). In some embodiments, the muscle-targeting agent is covalently linked to the

molecular payload. In some embodiments, the muscle-targeting agent is non-covalently linked to the molecular payload.

[000274] In some embodiments, the muscle-targeting agent is a substrate of an organic cation/carnitine transporter (OCTN2), which is a sodium ion-dependent, high affinity carnitine transporter. In some embodiments, the muscle-targeting agent is carnitine, mildronate, acetylcarnitine, or any derivative thereof that binds to OCTN2. In some embodiments, the carnitine, mildronate, acetylcarnitine, or derivative thereof is covalently linked to the molecular payload (e.g., oligonucleotide payload).

[000275] A muscle-targeting agent may be a protein that is protein that exists in at least one soluble form that targets muscle cells. In some embodiments, a muscle-targeting protein may be hemojuvelin (also known as repulsive guidance molecule C or hemochromatosis type 2 protein), a protein involved in iron overload and homeostasis. In some embodiments, hemojuvelin may be full length or a fragment, or a mutant with at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity to a functional hemojuvelin protein. In some embodiments, a hemojuvelin mutant may be a soluble fragment, may lack a N-terminal signaling, and/or (e.g., and) lack a C-terminal anchoring domain. In some embodiments, hemojuvelin may be annotated under GenBank RefSeq Accession Numbers NM_001316767.1, NM_145277.4, NM_202004.3, NM_213652.3, or NM_213653.3. It should be appreciated that a hemojuvelin may be of human, non-human primate, or rodent origin.

B. Molecular Payloads

[000276] Some aspects of the disclosure provide molecular payloads, e.g., for modulating a biological outcome, e.g., the transcription of a DNA sequence, the splicing and processing of a RNA sequence, the expression of a protein, or the activity of a protein. In some embodiments, a molecular payload is linked to, or otherwise associated with a muscle-targeting agent. In some embodiments, such molecular payloads are capable of targeting to a muscle cell, e.g., via specifically binding to a nucleic acid or protein in the muscle cell following delivery to the muscle cell by an associated muscle-targeting agent. It should be appreciated that various types of muscle-targeting agents may be used in accordance with the disclosure. For example, the molecular payload may comprise, or consist of, an oligonucleotide (e.g., antisense oligonucleotide), a peptide (e.g., a peptide that binds a nucleic acid or protein associated with disease in a muscle cell), a protein (e.g., a protein that binds a nucleic acid or protein associated with disease in a muscle cell), or a small molecule (e.g., a small molecule that modulates the function of a nucleic acid or protein associated with disease in a muscle

cell). In some embodiments, the molecular payload is an oligonucleotide that comprises a strand having a region of complementarity to a mutated DMD allele. Exemplary molecular payloads are described in further detail herein, however, it should be appreciated that the exemplary molecular payloads provided herein are not meant to be limiting.

i. Oligonucleotides

[000277] Any suitable oligonucleotide may be used as a molecular payload, as described herein. In some embodiments, the oligonucleotide may be designed to induce exon skipping, *e.g.*, EXONDYS 51 oligonucleotide (Sarepta Therapeutics, Inc.), which comprises SEQ ID NO: 343 (CUCCAACAUCAAGGAAGAUGGCAUUUCUAG); WVE-210201 (Wave Life Sciences), which comprises SEQ ID NO: 334 (UCAAGGAAGAUGGCAUUUCU); Casimersen (Sarepta Therapeutics, Inc.), which comprises SEQ ID NO: 302 (CAAUGCCAUCCUGGAGUUCCUG); or Golodirsen (Sarepta Therapeutics, Inc.), which comprises SEQ ID NO: 380 (GUUGCCUCCGGUUCUGAAGGUGUUC). In some embodiments, the oligonucleotide may be designed to induce exon skipping, *e.g.*, viltolarsen (NS Pharma, Inc.), which comprises SEQ ID NO: 2257 (CCTCCGGTTCTGAAGGTGTTC) or renadirsen (Daiichi Sankyo Company), which comprises SEQ ID NO: 2252 (CGCUGCCCAAUGCCAUCC). In some embodiments, the oligonucleotide comprises a sequence or portion thereof (*e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more consecutive nucleosides thereof) of a sequence provided in Table 10, and/or the oligonucleotide comprises a region of complementarity to a target sequence provided in Table 10. Any one or more of the thymine bases (T's) in any one of the oligonucleotides provided herein (e.g., the oligonucleotides listed in Table 10) may optionally be uracil bases (U's), and/or any one or more of the U's in the oligonucleotides provided herein may optionally be T's.

Table 10. Examples of oligonucleotide molecular payloads

Name	SEQ ID NO:	Antisense Sequence†	SEQ ID NO:	Antisense Sequence†	SEQ ID NO:	Target Sequence†
EXONDYS 51	343	CUCCAACAUCAAGGAAGAUGGCAUUUCUAG	745	CTCCAACATCAAGGAAGATGGCATTTCTAG	1550	CTAGAAATGCCATCTTCCTTGATGTTGGAG
WVE-210201	334	UCAAGGAAGAUGGCAUUUCU	2254	TCAAGGAAGATGGCATTTCT	2259	AGAAATGCCATCTTCCTTGA
Casimersen	302	CAAUGCCAUCCUGGAGUUCCUG	2255	CAATGCCATCCTGGAGTTCCTG	2260	CAGGAACTCCAGGATGGCATTG
Golodirsen	380	GUUGCCUCCGGUUCUGAAGGUGUUC	2256	GTTGCCTCCGGTTCTGAAGGTGTTC	2261	GAACACCTTCAGAACCGGAGGCAAC

Viltolarsen	2251	CCUCCGGUUCUGAAGGUGUUC	2257	CCTCCGGTTCTGAAGGTGTTC	2262	GAACACCTTCAGAACCGGAGG
Renadirsen	2252	CGCUGCCCAAUGCCAUCC	2258	CGCTGCCCAATGCCATCC	2263	GGATGGCATTGGGCAGCG

† *Each thymine base (T) in any one of the oligonucleotides and/or target sequences provided in Table 10 may independently and optionally be replaced with a uracil base (U), and/or each U may independently and optionally be replaced with a T. Target sequences listed in Table 10 contain Ts, but binding of a DMD-targeting oligonucleotide to RNA and/or DNA is contemplated.*

[000278] In some embodiments, the oligonucleotide may be designed to cause degradation of an mRNA (e.g., the oligonucleotide may be a gapmer, an siRNA, a ribozyme or an aptamer that causes degradation). In some embodiments, the oligonucleotide may be designed to block translation of an mRNA (e.g., the oligonucleotide may be a mixmer, an siRNA or an aptamer that blocks translation). In some embodiments, an oligonucleotide may be designed to cause degradation and block translation of an mRNA. In some embodiments, the oligonucleotide may be designed to promote stability of an mRNA. In some embodiments, the oligonucleotide may be designed to promote translation of an mRNA. In some embodiments, an oligonucleotide may be designed to promote stability and promote translation of an mRNA. In some embodiments, an oligonucleotide may be a guide nucleic acid (e.g., guide RNA) for directing activity of an enzyme (e.g., a gene editing enzyme). In some embodiments, a guide nucleic acid may direct an enzyme to delete the entirety or a part of a mutated DMD allele (e.g., to facilitate in-frame exon skipping). In some embodiments, the oligonucleotide may be designed to target repressive regulators of DMD expression, *e.g.*, miR-31. Other examples of oligonucleotides are provided herein. It should be appreciated that, in some embodiments, oligonucleotides in one format (e.g., antisense oligonucleotides) may be suitably adapted to another format (e.g., siRNA oligonucleotides) by incorporating functional sequences (e.g., antisense strand sequences) from one format to the other format.

[000279] Examples of oligonucleotides useful for targeting DMD are provided in U.S. Patent Application Publication US20100130591A1, published on May 27, 2010, entitled "MULTIPLE EXON SKIPPING COMPOSITIONS FOR DMD"; U.S. Patent No. 8,361,979, issued January 29, 2013, entitled "MEANS AND METHOD FOR INDUCING EXON-SKIPPING"; U.S. Patent Application Publication 20120059042, published March 8, 2012, entitled "METHOD FOR EFFICIENT EXON (44) SKIPPING IN DUCHENNE MUSCULAR DYSTROPHY AND ASSOCIATED MEANS; U.S. Patent Application Publication 20140329881, published November 6, 2014, entitled "EXON SKIPPING COMPOSITIONS FOR TREATING MUSCULAR DYSTROPHY"; U.S. Patent No. 8,232,384, issued July 31, 2012, entitled "ANTISENSE OLIGONUCLEOTIDES FOR INDUCING EXON SKIPPING

AND METHODS OF USE THEREOF"; U.S. Patent Application Publication 20120022134A1, published January 26, 2012, entitled "METHODS AND MEANS FOR EFFICIENT SKIPPING OF EXON 45 IN DUCHENNE MUSCULAR DYSTROPHY PRE-MRNA; U.S. Patent Application Publication 20120077860, published March 29, 2012, entitled "ADENO-ASSOCIATED VIRAL VECTOR FOR EXON SKIPPING IN A GENE ENCODING A DISPENSABLE DOMAN PROTEIN"; U.S. Patent No. 8,324,371, issued December 4, 2012, entitled "OLIGOMERS"; U.S. Patent No. 9,078,911, issued July 14, 2015, entitled "ANTISENSE OLIGONUCLEOTIDES"; U.S. Patent No. 9,079,934, issued July 14, 2015, entitled "ANTISENSE NUCLEIC ACIDS"; U.S. Patent No. 9,034,838, issued May 19, 2015, entitled "MIR-31 IN DUCHENNE MUSCULAR DYSTROPHY THERAPY"; and International Patent Publication WO2017062862A3, published April 13, 2017, entitled "OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF"; the contents of each of which are incorporated herein in their entireties.

[000280] Table 14 provides non-limiting examples of sequences of oligonucleotides that are useful for targeting DMD, *e.g.*, for exon skipping. In some embodiments, an oligonucleotide may comprise any sequence provided in Table 14.

Table 14 –Oligonucleotide sequences for targeting DMD.

EXON	SEQ ID NO:	SEQUENCE
8	151	CUUCCUGGAUGGCUUCAAU
8	152	GUACAUUAAGAUGGACUUC
8	153	UAUCUGGAUAGGUGGUAUCAAGAUCUGUAA
8	154	AUGUAACUGAAAAUGUUCUUCUUUA
8	155	UGGAUAGGUGGUAUCAACAUCUGUAAGCAC
8	156	GAUAGGUGGUAUCAACAUCUGU
8	157	UAUCUGGAUAGGUGGUAUCAACAUCUGUAA
8	158	AAACUUGGAAGAGUGAUGUGAUGUA
8	159	GCUCACUUGUUGAGGCAAAACUUGGAA
8	160	GCCUUGGCAACAUUUCCACUUCCUG
8	161	UACACACUUUACCUGUUGAGAAUAG
8	162	GAUAGGUGGUAUCAACAUCUGUAA
8	163	GAUAGGUGGUAUCAACAUCUG
8	164	GAUAGGUGGUAUCAACAUCUGUAAG
8	165	GGUGGUAUCAACAUCUGUAA
8	166	GUAUCAACAUCUGUAAGCAC
23	167	CGGCUAAUUUCAGAGGGCGCUUUCUUNGAC
23	168	ACAGUGGUGCUGAGAUAGUAUAGGCC
23	169	UAGGCCACUUUGUUGCUCUUGC
23	170	UUCAGAGGGCGCUUUCUUC
23	171	GGCCAAACCUCGGCUUACCUGAAAU
23	172	GGCCAAACCUCGGCUUACCU

35	173	UCUUCAGGUGCACCUUCUGUUUCUCAAUCU
35	174	UCUGUGAUACUCUUCAGGUGCACCUUCUGU
35	175	UCUUCUGCUCGGGAGGUGACA
35	176	CCAGUUACUAUUCAGAAGAC
35	177	UCUUCAGGUGCACCUUCUGU
43	178	UGCUGCUGUCUUCUUGCU
43	179	UUGUUAACUUUUUCCCAUU
43	180	UGUUAACUUUUUCCCAUUGG
43	181	CAUUUUGUUAACUUUUUCCC
43	182	CUGUAGCUUCACCCUUUCC
43	183	GAGAGCUUCCUGUAGCUUCACCCUUU
43	184	UCCUGUAGCUUCACCCUUUCCACAGGCG
43	185	UGUGUUACCUACCCUUGUCG
43	186	UAGACUAUCUUUUAUAUUCUGUAAUAU
43	187	GAGAGCUUCCUGUAGCUUCACCCUUUCCA
43	188	UUCCUGUAGCUUCACCCUUUCCACAGGCGUU
43	189	AGCUUCCUGUAGCUUCACCCUUU
43	190	GGAGAGAGCUUCCUGUAGCUUCACCCUUU
43	191	GAGAGCUUCCUGUAGCUUCACCC
43	192	UAUGUGUUACCUACCCUUGUCGGUC
43	193	GGAGAGAGCUUCCUGUAGCU
43	194	UCACCCUUUCCACAGGCGUUGCA
43	195	GCUGGGAGAGAGCUUCCUGUAGCUUCAC
43	196	UGUUACCUACCCUUGUCGGUCCUUGUAC
43	197	CUGCUGUCUUCUUGCUAUGAAUAAUGUC
43	198	GGCGUUGCACUUUGCAAUGCUGCUGUCU
43	199	UUGGAAAUCAAGCUGGGAGAGAGCUUCC
43	200	CUACCCUUGUCGGUCCUUGUACAUUUUG
43	201	GUCAAUCCGACCUGAGCUUUGUUGUAGA
43	202	CUUGCUAUGAAUAAUGUCAAUCCGACC
43	203	UAUAUGUGUUACCUACCCUUGUCGGUCC
43	204	AAUCAGCUGGGAGAGAGCUUCCUGUAGCU
43	205	UCGUUCUUCUGUCGUCGUAACGUUUC
44	206	UUUGUGUCUUUCUGAGAAAC
44	207	AAAGACUUACCUUAAGAUAC
44	208	AUCUGUCAAAUCGCCUGCAG
44	209	CGCCGCCAUUUCUCAACAG
44	210	UUUGUAUUUAGCAUGUUCCC
44	211	CCGCCAUUUCUCAACAG
44	212	UUCUCAGGAAUUUGUGUCUUU
44	213	GACAACUCUUU
44	214	UCAGCUUCUGUUAGCCACUG
44	215	UGUUCAGCUUCUGUUAGCCACUGA
44	216	CUGUUCAGCUUCUGUUAGCCACUGAUU
44	217	UUCUCAACAGAUCUGUCAAAUCGCCUGCAG

44	218	GCCACUGAUUAAAUAUCUUUAUAUC
44	219	UCUGUUAGCCACUGAUUAAAUAUCUUUAUA
44	220	GAGAAACUGUUCAGCUUCUGUUAGCCACUGA
44	221	UCUUUCUGAGAAACUGUUCAGCUUCUGUUAG
44	222	CAGAUCUGUCAAAUCGCCUGCAGGUA
44	223	CAACAGAUCUGUCAAAUCGCCUGCAG
44	224	AAACUGUUCAGCUUCUGUUAGCCACUGAUUAAA
44	225	GAAACUGUUCAGCUUCUGUUAGCCACUGAUU
44	226	AAACUGUUCAGCUUCUGUUAGCCACUGA
44	227	UGAGAAACUGUUCAGCUUCUGUUAGCCA
44	228	UUCUGAGAAACUGUUCAGCUUCUGUUAGCCAC
44	229	UUCUGAGAAACUGUUCAGCUUCUGUU
44	230	GAUCUGUCAAAUCGCCUGCAGGUAA
44	231	AUAAUGAAAACGCCGCCAUUUCUCA
44	232	AAACUGUUCAGCUUCUGUUAGCCAC
44	233	UUGUGUCUUUCUGAGAAACUGUUCA
44	234	CCAAUUCUCAGGAAUUUGUGUCUUU
44	235	AUCGCCUGCAGGUAAAAGCAUAUGG
44	236	UGAAAACGCCGCCAUUUCUCAACAGAUCUG
44	237	CAUAAUGAAAACGCCGCCAUUUCUCAACAG
44	238	UGUUCAGCUUCUGUUAGCCACUGAUUAAAU
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44	243	GAUCUGUCAAAUCGCCUGCAGG
44	244	GAUCUGUCAAAUCGCCUGCAG
44	245	CAGAUCUGUCAAAUCGCCUGCAGGU
44	246	CAGAUCUGUCAAAUCGCCUGCAG
44	247	GUGUCUUUCUGAGAAACUGUUCAGC
44	248	GAGAAACUGUUCAGCUUCUGUUAGCCAC
44	249	GAAACUGUUCAGCUUCUGUUAGCCACUG
44	250	CUGUUCAGCUUCUGUUAGCCACUG
44	251	AUCUGUCAAAUCGCCUGCAGGUAAAAG
44	252	GAUCUGUCAAAUCGCCUGCAGGUAAAAGC
44	253	CACCGAUUGUCUUCGA
44	254	CCCUUGUACGAUUUAUG
44	255	UCUGUGUUUAAGGACUCU
45	256	GCUGAAUUAUUUCUUCCCC
45	257	UUUUUCUGUCUGACAGCUG
45	258	UCUGUUUUUGAGGAUUGC
45	259	CCACCGCAGAUUCAGGC
45	260	GCCCAAUGCCAUCCUGG
45	261	UUUGCAGACCUCCUGCC
45	262	CAGUUUGCCGCUGCCCA

45	263	GUUGCAUUCAAUGUUCUGAC
45	264	AUUUUUCCUGUAGAAUACUGG
45	265	GCUGCCCAAUGCGAUCCUGGAGUUCCUGUAAGAU
45	266	GCUGCCCAAUGCCAUCCUGGAGUUCCUG
45	267	GCUGCCCAAUGCCAUCCUGGAGUUCCUGUAA
45	268	CAAUGCCAUCCUGGAGUUCCUGUAAGAUACC
45	269	GCUGCCCAAUGCCAUCCUGGAGUUCCUGUAAG
45	270	CCAAUGCCAUCCUGGAGUUCCUGUAAGAUA
45	271	UUGCCGCUGCCCAAUGCCAUCCUGGAGUUCCUGUAAGAU
45	272	GCUGCCCAAUGCCAUCCUGGAGUUCCUGUAAGAU
45	273	CAAUGCCAUCCUGGAGUUCCUGUAAGA
45	274	CAGUUUGCCGCUGCCCAAUGCCAUCC
45	275	CUUCCCCAGUUGCAUUCAAUGUUC
45	276	CUGGCAUCUGUUUUUGAGGAUUG
45	277	UUAGAUCUGUCGCCCUACCU
45	278	GCUGCCCAAUGCCAUCCUGGAGUUCCUGUAAGAUACCAA
45	279	GCCCAAUGCCAUCCUGGAGUUCCUGUAAGAUACC
45	280	CAUCCUGGAGUUCCUGUAAGAUACC
45	281	UGCCAUCCUGGAGUUCCUGUAAGAUACC
45	282	UGCCAUCCUGGAGUUCCUGUAAGAU
45	283	CAAUGCCAUCCUGGAGUUCCUGUAAGAU
45	284	GCCCAAUGCCAUCCUGGAGUUCCUGUAAGAU
45	285	GCCCAAUGCCAUCCUGGAGUUCCUGUAA
45	286	GCCGCUGCCCAAUGACAUCCUGGAGUUCCUGUAA
45	287	GCCAUCCUGGAGUUCCUGUAAGAUA
45	288	CCAAUGCCAUCCUGGAGUUCCUGUA
45	289	CUGACAACAGUUUGCCGCUGCCCAA
45	290	UUUGAGGAUUGCUGAAUUAUUUCUU
45	291	CAGUUUGCCGCUGCCCAAUGCCAUCCUGGA
45	292	UUGCCGCUGCCCAAUGCCAUCCUGGAGUUC
45	293	UUUGCCGCUGCCCAAUGCCAUCCUG
45	294	CCAAUGCCAUCCUGGAGUUCCU
45	295	CCCAAUGCCAUCCUGGAGUUCCUGUAAGA
45	296	CCGCUGCCCAAUGCCAUCCUGGAGUUCC
45	297	CCCAAUGCCAUCCUGGAGUUCCUGUAAGAU
45	298	CCGCUGCCCAAUGCCAUCCUGGAGUUCCUG
45	299	UGCCCAAUGCCAUCCUGGAGUUCCUGUAAG
45	300	CCCAAUGCCAUCCUGGAGUUCCUGUAAG
45	301	UGCCCAAUGCCAUCCUGGAGUUCCUGUA
45	302	CAAUGCCAUCCUGGAGUUCCUG
45	303	GCCGCUGCCCAAUGCCAUCCUGGAGUUCCUG
45	304	AUUAGAUCUGUCGCCCUACCUCUUUUUUC
45	305	UGUCGCCCUACCUCUUUUUUCUGUCUG
45	306	GCCCAAUGCCAUCCUGGAGUUCCUG
55	307	AGCCUCUCGCUCACUCACCCUGCAAAGGA

50	308	CCACUCAGAGCUCAGAUCUUCUAACUUCC
50	309	CUUCCACUCAGAGCUCAGAUCUUCUAA
50	310	GGGAUCCAGUAUACUUACAGGCUCC
50	311	CUCAGAGCUCAGAUCUU
50	312	GGCUGCUUUGCCCUC
50	313	CUCAGAUCUUCUAACUUCCUCUUUAAC
50	314	CUCAGAGCUCAGAUCUUCUAACUUCCUCU
50	315	CGCCUUCCACUCAGAGCUCAGAUCUUC
50	316	UCAGCUCUUGAAGUAAACGGUUUACCG
50	317	UUUGCCCUCAGCUCUUGAAGUAAACGG
50	318	GGCUGCUUUGCCCUCAGCUCUUGAAGU
50	319	CAGGAGCUAGGUCAGGCUGCUUUGCC
50	320	UCCAAUAGUGGUCAGUCCAGGAGCU
50	321	AAAGAGAAUGGGAUCCAGUAUACUUAC
50	322	AAAUAGCUAGAGCCAAAGAGAAUGGGA
50	323	GGCUGCUUUGCCCUCAGCUCUUGAAGUAAACGG
50	324	AGGCUGCUUUGCCCUCAGCUCUUGAAGUAA
50	325	GUCAGGCUGCUUUGCCCUCAGCUCUUGAAG
50	326	AGGUCAGGCUGCUUUGCCCUCAGCUCUUGA
50	327	CAGAGCUCAGAUCUUCUAACUUCCU
50	328	CUUACAGGCUCCAAUAGUGGUCAGU
50	329	AUGGGAUCCAGUAUACUUACAGGCU
50	330	AGAGAAUGGGAUCCAGUAUACUUAC
50	331	AACUUCCUCUUUAACAGAAAAGCAUAC
50	332	GAGCCUCUCGCUCACUCACCCUGCAAAGGA
51	333	CUCAUACCUUCUGCUUGAUGAUC
51	334	UCAAGGAAGAUGGCAUUUCU
51	335	GAAAGCCAGUCGGUAAGUUC
51	336	CACCCACCAUCACCC
51	337	CCUCUGUGAUUUUAUAACUUGAU
51	338	UGAUAUCCUCAAGGUCACCC
51	339	GGUACCUCCAACAUCAAGGAAGAUGGCAUU
51	340	AUUUCUAGUUUGGAGAUGGCAGUUUC
51	341	CAUCAAGGAAGAUGGCAUUUCUAGUU
51	342	GAGCAGGUACCUCCAACAUCAAGGAA
51	343	CUCCAACAUCAAGGAAGAUGGCAUUUCUAG
51	344	ACCAGAGUAACAGUCUGAGUAGGAG
51	345	CACCAGAGUAACAGUCUGAGUAGGA
51	346	UCACCAGAGUAACAGUCUGAGUAGG
51	347	GUCACCAGAGUAACAGUCUGAGUAG
51	348	ACCAGAGUAACAGUCUGAGUAGGAGC
51	349	UUCUGUCCAAGCCCGGUUGAAAUC
51	350	ACAUCAAGGAAGAUGGCAUUUCUAGUUUGG
51	351	ACAUCAAGGAAGAUGGCAUUUCUAG
51	352	AUCAUUUUUUCUCAUACCUUCUGCU

51	353	CACCCACCAUCACCCUCUGUG
51	354	AUCAUCUCGUUGAUAUCCUCAA
51	355	CUCCAACAUCAAGGAAGAUGGCAUUUCU
51	356	CAUCAAGGAAGAUGGCAUUUCUAGU
51	357	AUCAUUUUUUCUCAUACCUUCUGCUAGGAGCUAAAA
52	358	UUGCUGGUCUUGUUUUUC
52	359	CCGUAAUGAUUGUUCU
52	360	GCUGGUCUUGUUUUUCAA
52	361	UGGUCUUGUUUUUCAAAUUU
52	362	GUCUUGUUUUUCAAAUUUUG
52	363	CUUGUUUUUCAAAUUUUGGG
52	364	UGUUUUUCAAAUUUUGGGC
52	365	UCCAACUGGGGACGCCUCUGUUCCAAAUCCUGC
52	366	UCCUGCAUUGUUGCCUGUAAG
52	367	UCCAACUGGGGACGCCUCUGUUCCAAAUCC
52	368	ACUGGGGACGCCUCUGUUCCA
52	369	CCGUAAUGAUUGUUCUAGCC
52	370	UGUUAAAAAACUUACUUCGA
53	371	CUGUUGCCUCCGGUUCUG
53	372	UUGGCUCUGGCCUGUCCU
53	373	UUCAACUGUUGCCUCCGGUUCUGAAGGUGUUCU
53	374	UACUUCAUCCCACUGAUUCUGAAUU
53	375	CUGAAGGUGUUCUUGUACUUCAUCC
53	376	CUGUUGCCUCCGGUUCUGAAGGUGU
53	377	CUGUUGCCUCCGGUUCUGAAGGUGUUCUUG
53	378	CAACUGUUGCCUCCGGUUCUGAAGGUGUUC
53	379	UUGCCUCCGGUUCUGAAGGUGUUCUUGUAC
53	380	GUUGCCUCCGGUUCUGAAGGUGUUC
53	381	CUCCGGUUCUGAAGGUGUUCUUG
53	382	CUCCGGUUCUGAAGGUGUUCUU
53	383	CUCCGGUUCUGAAGGUGUUCU
53	384	CUCCGGUUCUGAAGGUGUUC
53	385	CUCCGGUUCUGAAGGUGUU
53	386	CAUUCAACUGUUGCCUCCGGUUCUG
53	387	CUGUUGCCUCCGGUUCUGAAGGUG
53	388	CAUUCAACUGUUGCCUCCGGUUCUGAAGGUG
53	389	UACUAACCUUGGUUUCUGUGA
53	390	UGUAUAGGGACCCUCCUUCCAUGACUC
53	391	CUAACCUUGGUUUCUGUGAUUUUCU
53	392	GGUAUCUUUGAUACUAACCUUGGUUUC
53	393	AUUCUUUCAACUAGAAUAAAAG
53	394	GAUUCUGAAUUCUUUCAACUAGAAU
53	395	AUCCCACUGAUUCUGAAUUC
53	396	AACCGAGACCGGACAGGAUUCU
53	397	GGAAGCUAAGGAAGAAGCUGAGCAGG

55	398	CUGUUGCAGUAAUCUAUGAG
55	399	UGCCAUUGUUUCAUCAGCUCUUU
55	400	UGCAGUAAUCUAUGAGUUUC
55	401	UCCUGUAGGACAUUGGCAGU
55	131	GAGUCUUCUAGGAGCCUU

[000281] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) targets a region of a DMD RNA (*e.g.*, the Dp427m transcript of SEQ ID NO: 2239). In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) comprises a region of complementarity to a DMD RNA (*e.g.*, the Dp427m transcript of SEQ ID NO: 2239). In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) comprises a region of complementarity to an exon of a DMD RNA (*e.g.*, any one of SEQ ID NOs: 2240-2250). Examples of DMD RNA sequences and exon sequences are provided below.

[000282] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, mRNA (NCBI Reference Sequence: NM_004006.2)

TCCTGGCATCAGTTACTGTGTTGACTCACTCAGTGTTGGGATCACTCACTTTCCCCCTACAGGACTCA
GATCTGGGAGGCAATTACCTTCGGAGAAAAACGAATAGGAAAAACTGAAGTGTTACTTTTTTTAAA
GCTGCTGAAGTTTGTTGGTTTCTCATTGTTTTTAAGCCTACTGGAGCAATAAAGTTTGAAGAACTTTT
ACCAGGTTTTTTTTATCGCTGCCTTGATATACACTTTTCAAAATGCTTTGGTGGGAAGAAGTAGAGG
ACTGTTATGAAAGAGAAGATGTTCAAAAGAAAACATTCACAAAATGGGTAAATGCACAATTTTCTA
AGTTTGGGAAGCAGCATATTGAGAACCTCTTCAGTGACCTACAGGATGGGAGGCGCCTCCTAGACCT
CCTCGAAGGCCTGACAGGGCAAAAACTGCCAAAAGAAAAAGGATCCACAAGAGTTCATGCCCTGAA
CAATGTCAACAAGGCACTGCGGGTTTTGCAGAACAATAATGTTGATTTAGTGAATATTGGAAGTACT
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GCTGGGTCCGACAATCAACTCGTAATTATCCACAGGTTAATGTAATCAACTTCACCACCAGCTGGTC
TGATGGCCTGGCTTTGAATGCTCTCATCCATAGTCATAGGCCAGACCTATTTGACTGGAATAGTGTG
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GCTATGCCTACACACAGGCTGCTTATGTCACCACCTCTGACCCTACACGGAGCCCATTTCCTTCACA
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CAAGGAGAGATTTCTAATGATGTGGAAGTGGTGAAAGACCAGTTTCATACTCATGAGGGGTACATG
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CAAGAAGATCTAGAACAAGAACAAGTCAGGGTCAATTCTCTCACTCACATGGTGGTGGTAGTTGAT
GAATCTAGTGGAGATCACGCAACTGCTGCTTTGGAAGAACAACTTAAGGTATTGGGAGATCGATGG
GCAAACATCTGTAGATGGACAGAAGACCGCTGGGTTCTTTTACAAGACATCCTTCTCAAATGGCAAC
GTCTTACTGAAGAACAGTGCCTTTTTAGTGCATGGCTTTCAGAAAAAGAAGATGCAGTGAACAAGAT
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TGGGAAAAAGTTAACAAAATGTACAAGGACCGACAAGGGCGATTTGACAGATCTGTTGAGAAATGG
CGGCGTTTTCATTATGATATAAAGATATTTAATCAGTGGCTAACAGAAGCTGAACAGTTTCTCAGAA
AGACACAAATTCCTGAGAATTGGGAACATGCTAAATACAAATGGTATCTTAAGGAACTCCAGGATG
GCATTGGGCAGCGGCAAACTGTTGTCAGAACATTGAATGCAACTGGGGAAGAAATAATTCAGCAAT
CCTCAAAAACAGATGCCAGTATTCTACAGGAAAAATTGGGAAGCCTGAATCTGCGGTGGCAGGAGG
TCTGCAAACAGCTGTCAGACAGAAAAAAGAGGCTAGAAGAACAAAAGAATATCTTGTCAGAATTTC
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ACCTGGAAAAGAGCAGCAACTAAAAGAAAAGCTTGAGCAAGTCAAGTTACTGGTGGAAGAGTTGCC
CCTGCGCCAGGGAATTCTCAAACAATTAAATGAAACTGGAGGACCCGTGCTTGTAAGTGCTCCCATA
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AGTTGGAAATTTATAACCAACCAAACCAAGAAGGACCATTTGACGTTCAGGAAACTGAAATAGCAG
TTCAAGCTAAACAACCGGATGTGGAAGAGATTTTGTCTAAAGGGCAGCATTTGTACAAGGAAAAAC
CAGCCACTCAGCCAGTGAAGAGGAAGTTAGAAGATCTGAGCTCTGAGTGGAAGGCGGTAAACCGTT
TACTTCAAGAGCTGAGGGCAAAGCAGCCTGACCTAGCTCCTGGACTGACCACTATTGGAGCCTCTCC
TACTCAGACTGTTACTCTGGTGACACAACCTGTGGTTACTAAGGAAACTGCCATCTCCAAACTAGAA
ATGCCATCTTCCTTGATGTTGGAGGTACCTGCTCTGGCAGATTTCAACCGGGCTTGGACAGAACTTA
CCGACTGGCTTTCTCTGCTTGATCAAGTTATAAAATCACAGAGGGTGATGGTGGGTGACCTTGAGGA
TATCAACGAGATGATCATCAAGCAGAAGGCAACAATGCAGGATTTGGAACAGAGGCGTCCCCAGTT
GGAAGAACTCATTACCGCTGCCCAAAATTTGAAAAACAAGACCAGCAATCAAGAGGCTAGAACAAT
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GAGGCAACAGTTGAATGAAATGTTAAAGGATTCAACACAATGGCTGGAAGCTAAGGAAGAAGCTG
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CAATCCAAAAGAAAATCACAGAAACCAAGCAGTTGGCCAAAGACCTCCGCCAGTGGCAGACAAAT
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CTGGCTTACAGAAGCTGAAACAACTGCCAATGTCCTACAGGATGCTACCCGTAAGGAAAGGCTCCT
AGAAGACTCCAAGGGAGTAAAAGAGCTGATGAAACAATGGCAAGACCTCCAAGGTGAAATTGAAG
CTCACACAGATGTTTATCACAACCTGGATGAAAACAGCCAAAAAATCCTGAGATCCCTGGAAGGTT
CCGATGATGCAGTCCTGTTACAAAGACGTTTGGATAACATGAACTTCAAGTGGAGTGAACTTCGGAA
AAAGTCTCTCAACATTAGGTCCCATTTGGAAGCCAGTTCTGACCAGTGGAAGCGTCTGCACCTTTCT
CTGCAGGAACTTCTGGTGTGGCTACAGCTGAAAGATGATGAATTAAGCCGGCAGGCACCTATTGGA
GGCGACTTTCCAGCAGTTCAGAAGCAGAACGATGTACATAGGGCCTTCAAGAGGGAATTGAAAACT
AAAGAACCTGTAATCATGAGTACTCTTGAGACTGTACGAATATTTCTGACAGAGCAGCCTTTGGAAG
GACTAGAGAAACTCTACCAGGAGCCCAGAGAGCTGCCTCCTGAGGAGAGAGCCCAGAATGTCACTC
GGCTTCTACGAAAGCAGGCTGAGGAGGTCAATACTGAGTGGGAAAAATTGAACCTGCACTCCGCTG
ACTGGCAGAGAAAAATAGATGAGACCCTTGAAAGACTCCAGGAACTTCAAGAGGCCACGGATGAG
CTGGACCTCAAGCTGCGCCAAGCTGAGGTGATCAAGGGATCCTGGCAGCCCGTGGGCGATCTCCTC
ATTGACTCTCTCCAAGATCACCTCGAGAAAGTCAAGGCACTTCGAGGAGAAATTGCGCCTCTGAAA
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TCTTGAGCCTGTCAGCTGCATGTGATGCCTTGGACCAGCACAACCTCAAGCAAAATGACCAGCCCAT
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TTGGTCAACGTCCCTCTCTGCGTGGATATGTGTCTGAACTGGCTGCTGAATGTTTATGATACGGGAC
GAACAGGGAGGATCCGTGTCCTGTCTTTTAAAACTGGCATCATTTCCCTGTGTAAAGCACATTTGGA
AGACAAGTACAGATACCTTTTCAAGCAAGTGGCAAGTTCAACAGGATTTTGTGACCAGCGCAGGCT
GGGCCTCCTTCTGCATGATTCTATCCAAATTCCAAGACAGTTGGGTGAAGTTGCATCCTTTGGGGGC
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CCCGAATGGGCTACCTGCCAGTGCAGACTGTCTTAGAGGGGGACAACATGGAAACTCCCGTTACTCT
GATCAACTTCTGGCCAGTAGATTCTGCGCCTGCCTCGTCCCCTCAGCTTTCACACGATGATACTCATT
CACGCATTGAACATTATGCTAGCAGGCTAGCAGAAATGGAAAACAGCAATGGATCTTATCTAAATG
ATAGCATCTCTCCTAATGAGAGCATAGATGATGAACATTTGTTAATCCAGCATTACTGCCAAAGTTT
GAACCAGGACTCCCCCCTGAGCCAGCCTCGTAGTCCTGCCCAGATCTTGATTTCCTTAGAGAGTGAG
GAAAGAGGGGAGCTAGAGAGAATCCTAGCAGATCTTGAGGAAGAAAACAGGAATCTGCAAGCAGA
ATATGACCGTCTAAAGCAGCAGCACGAACATAAAGGCCTGTCCCCACTGCCGTCCCCTCCTGAAATG
ATGCCCACCTCTCCCCAGAGTCCCCGGGATGCTGAGCTCATTGCTGAGGCCAAGCTACTGCGTCAAC
ACAAAGGCCGCCTGGAAGCCAGGATGCAAATCCTGGAAGACCACAATAAACAGCTGGAGTCACAGT
TACACAGGCTAAGGCAGCTGCTGGAGCAACCCCAGGCAGAGGCCAAAGTGAATGGCACAACGGTGT
CCTCTCCTTCTACCTCTCTACAGAGGTCCGACAGCAGTCAGCCTATGCTGCTCCGAGTGGTTGGCAGT
CAAACTTCGGACTCCATGGGTGAGGAAGATCTTCTCAGTCCTCCCCAGGACACAAGCACAGGGTTA
GAGGAGGTGATGGAGCAACTCAACAACTCCTTCCCTAGTTCAAGAGGAAGAAATACCCCTGGAAAG
CCAATGAGAGAGGACACAATGTAGGAAGTCTTTTCCACATGGCAGATGATTTGGGCAGAGCGATGG
AGTCCTTAGTATCAGTCATGACAGATGAAGAAGGAGCAGAATAAATGTTTTACAACTCCTGATTCCC
GCATGGTTTTTATAATATTCATACAACAAAGAGGATTAGACAGTAAGAGTTTACAAGAAATAAATCT
ATATTTTTGTGAAGGGTAGTGGTATTATACTGTAGATTTCAGTAGTTTCTAAGTCTGTTATTGTTTTGT
TAACAATGGCAGGTTTTACACGTCTATGCAATTGTACAAAAAAGTTATAAGAAAACTACATGTAAA
ATCTTGATAGCTAAATAACTTGCCATTTCTTTATATGGAACGCATTTTGGGTTGTTTAAAAATTTATA
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GTTTTGCATCCTTTTGGCGTGATATCCATATGAAATTCATGGCTTTTTCTTTTTTTGCATATTAAAGAT
AAGACTTCCTCTACCACCACACCAAATGACTACTACACACTGCTCATTTGAGAACTGTCAGCTGAGT
GGGGCAGGCTTGAGTTTTCATTTCATATATCTATATGTCTATAAGTATATAAATACTATAGTTATATA
GATAAAGAGATACGAATTTCTATAGACTGACTTTTTCCATTTTTTAAATGTTCATGTCACATCCTAAT
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GCCGGAAGCCAGGAGGAAACTACACCACACTAAAACATTGTCTACAGCTCCAGATGTTTCTCATTTT
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GAATACACAGGACTTATTATATCAGAGTGAGTAATCGGTTGGTTGGTTGATTGATTGATTGATTGAT
ACATTCAGCTTCCTGCTGCTAGCAATGCCACGATTTAGATTTAATGATGCTTCAGTGGAAATCAATC
AGAAGGTATTCTGACCTTGTGAACATCAGAAGGTATTTTTTAACTCCCAAGCAGTAGCAGGACGATG
ATAGGGCTGGAGGGCTATGGATTCCCAGCCCATCCCTGTGAAGGAGTAGGCCACTCTTTAAGTGAA
GGATTGGATGATTGTTCATAATACATAAAGTTCTCTGTAATTACAACTAAATTATTATGCCCTCTTCT
CACAGTCAAAAGGAACTGGGTGGTTTGGTTTTTGTTGCTTTTTTAGATTTATTGTCCCATGTGGGATG
AGTTTTTAAATGCCACAAGACATAATTTAAAATAAATAAACTTTGGGAAAAGGTGTAAAACAGTAG
CCCCATCACATTTGTGATACTGACAGGTATCAACCCAGAAGCCCATGAACTGTGTTTCCATCCTTTGC
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CAAAAAAGAGGAGAAAAGCTCAAGAGGAAAAGCTAAGGACTGGTAGGAAAAAGCTTTACTCTTTC
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TGCAAATCTGTTACCTCTGACATCAAGTGTAATTAGCTTTTGGAGAGTGGGCTGACATCAAGTGTAA
TTAGCTTTTGGAGAGTGGGTTTTGTCCATTATTAATAATTAATTAATTAACATCAAACACGGCTTCTC
ATGCTATTTCTACCTCACTTTGGTTTTGGGGTGTTCCTGATAATTGTGCACACCTGAGTTCACAGCTT
CACCACTTGTCCATTGCGTTATTTTCTTTTTCCTTTATAATTCTTTCTTTTTCCTTCATAATTTTCAAAA
GAAAACCCAAAGCTCTAAGGTAACAAATTACCAAATTACATGAAGATTTGGTTTTTGTCTTGCATTT
TTTTCCTTTATGTGACGCTGGACCTTTTCTTTACCCAAGGATTTTTAAAACTCAGATTTAAAACAAGG
GGTTACTTTACATCCTACTAAGAAGTTTAAGTAAGTAAGTTTCATTCTAAAATCAGAGGTAAATAGA
GTGCATAAATAATTTTGTTTTAATCTTTTTGTTTTTCTTTTAGACACATTAGCTCTGGAGTGAGTCTGT
CATAATATTTGAACAAAAATTGAGAGCTTTATTGCTGCATTTTAAGCATAATTAATTTGGACATTATT
TCGTGTTGTGTTCTTTATAACCACCAAGTATTAAACTGTAAATCATAATGTAACTGAAGCATAAACA
TCACATGGCATGTTTTGTCATTGTTTTCAGGTACTGAGTTCTTACTTGAGTATCATAATATATTGTGTT
TTAACACCAACACTGTAACATTTACGAATTATTTTTTTAAACTTCAGTTTTACTGCATTTTCACAACA
TATCAGACTTCACCAAATATATGCCTTACTATTGTATTATAGTACTGCTTTACTGTGTATCTCAATAA
AGCACGCAGTTATGTTAC (SEQ ID NO: 2239)

[000283] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 8 (nucleotide positions 894-1075 of NCBI Reference Sequence: NM_004006.2)

ATGTTGATACCACCTATCCAGATAAGAAGTCCATCTTAATGTACATCACATCACTCTTCCAAGTTTTG
CCTCAACAAGTGAGCATTGAAGCCATCCAGGAAGTGGAAATGTTGCCAAGGCCACCTAAAGTGACT
AAAGAAGAACATTTTCAGTTACATCATCAAATGCACTATTCTCAACAG (SEQ ID NO: 2240)

[000284] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 23

(nucleotide positions 3194-3406 of NCBI Reference Sequence: NM_004006.2)

GCTTTACAAAGTTCTCTGCAAGAGCAACAAAGTGGCCTATACTATCTCAGCACCACTGTGAAAGAGA
TGTCGAAGAAAGCGCCCTCTGAAATTAGCCGGAAATATCAATCAGAATTTGAAGAAATTGAGGGAC
GCTGGAAGAAGCTCTCCTCCCAGCTGGTTGAGCATTGTCAAAAGCTAGAGGAGCAAATGAATAAAC
TCCGAAAAATTCAG (SEQ ID NO: 2241)

[000285] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 43

(nucleotide positions 6362-6534 of NCBI Reference Sequence: NM_004006.2)

AATATAAAAGATAGTCTACAACAAAGCTCAGGTCGGATTGACATTATTCATAGCAAGAAGACAGCA
GCATTGCAAAGTGCAACGCCTGTGGAAAGGGTGAAGCTACAGGAAGCTCTCTCCCAGCTTGATTTCC
AATGGGAAAAAGTTAACAAAATGTACAAGGACCGACAAGG (SEQ ID NO: 2242)

[000286] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 44

(nucleotide positions 6535-6682 of NCBI Reference Sequence: NM_004006.2)

GCGATTTGACAGATCTGTTGAGAAATGGCGGCGTTTTCATTATGATATAAAGATATTTAATCAGTGG
CTAACAGAAGCTGAACAGTTTCTCAGAAAGACACAAATTCCTGAGAATTGGGAACATGCTAAATAC
AAATGGTATCTTAAG (SEQ ID NO: 2243)

[000287] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 45

(nucleotide positions 6683-6858 of NCBI Reference Sequence: NM_004006.2)

GAACTCCAGGATGGCATTGGGCAGCGGCAAACTGTTGTCAGAACATTGAATGCAACTGGGGAAGAA
ATAATTCAGCAATCCTCAAAAACAGATGCCAGTATTCTACAGGAAAAATTGGGAAGCCTGAATCTG
CGGTGGCAGGAGGTCTGCAAACAGCTGTCAGACAGAAAAAAGAG (SEQ ID NO: 2244)

[000288] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 46

(nucleotide positions 6859-7006 of NCBI Reference Sequence: NM_004006.2)

GCTAGAAGAACAAAAGAATATCTTGTCAGAATTTCAAAGAGATTTAAATGAATTTGTTTTATGGTTG
GAGGAAGCAGATAACATTGCTAGTATCCCACTTGAACCTGGAAAAGAGCAGCAACTAAAAGAAAA
GCTTGAGCAAGTCAAG (SEQ ID NO: 2245)

[000289] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 50

(nucleotide positions 7445-7553 of NCBI Reference Sequence: NM_004006.2)

AGGAAGTTAGAAGATCTGAGCTCTGAGTGGAAGGCGGTAAACCGTTTACTTCAAGAGCTGAGGGCA
AAGCAGCCTGACCTAGCTCCTGGACTGACCACTATTGGAGCCT (SEQ ID NO: 2246)

[000290] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 51

(nucleotide positions 7554-7786 of NCBI Reference Sequence: NM_004006.2)

CTCCTACTCAGACTGTTACTCTGGTGACACAACCTGTGGTTACTAAGGAAACTGCCATCTCCAAACT
AGAAATGCCATCTTCCTTGATGTTGGAGGTACCTGCTCTGGCAGATTTCAACCGGGCTTGGACAGAA
CTTACCGACTGGCTTTCTCTGCTTGATCAAGTTATAAAATCACAGAGGGTGATGGTGGGTGACCTTG
AGGATATCAACGAGATGATCATCAAGCAGAAG (SEQ ID NO: 2247)

[000291] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 52 (nucleotide positions 7787-7904 of NCBI Reference Sequence: NM_004006.2)

GCAACAATGCAGGATTTGGAACAGAGGCGTCCCCAGTTGGAAGAACTCATTACCGCTGCCCAAAAT TTGAAAAACAAGACCAGCAATCAAGAGGCTAGAACAATCATTACGGATCGAA (SEQ ID NO: 2248)

[000292] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 53 (nucleotide positions 7905-8116 of NCBI Reference Sequence: NM_004006.2)

TTGAAAGAATTCAGAATCAGTGGGATGAAGTACAAGAACACCTTCAGAACCGGAGGCAACAGTTGA ATGAAATGTTAAAGGATTCAACACAATGGCTGGAAGCTAAGGAAGAAGCTGAGCAGGTCTTAGGAC AGGCCAGAGCCAAGCTTGAGTCATGGAAGGAGGGTCCCTATACAGTAGATGCAATCCAAAAGAAAA TCACAGAAACCAAG (SEQ ID NO: 2249)

[000293] *Homo sapiens* dystrophin (DMD), transcript variant Dp427m, exon 55 (nucleotide positions 8272-8461 of NCBI Reference Sequence: NM_004006.2)

GGTGAGTGAGCGAGAGGCTGCTTTGGAAGAAACTCATAGATTACTGCAACAGTTCCCCCTGGACCT GGAAAAGTTTCTTGCCTGGCTTACAGAAGCTGAAACAACTGCCAATGTCCTACAGGATGCTACCCGT AAGGAAAGGCTCCTAGAAGACTCCAAGGGAGTAAAAGAGCTGATGAAACAATGGCAA (SEQ ID NO: 2250)

[000294] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) targets an exonic splicing enhancer (ESE) sequence in DMD (e.g., an ESE sequence of exon 23, 44, 45, 46, 50, 51, 52, 53, or 55). In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) targets an exonic splicing enhancer (ESE) sequence in DMD (e.g., an ESE sequence of exon 8, 23, 43, 44, 45, 46, 50, 51, 52, 53, or 55). In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) targets an ESE sequence of DMD exon 51 (e.g., the ESEs listed in Table 15). In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) targets an ESE sequence of DMD exon 8, 23, 42, 44, 45, 46, 50, 52, 53, or 55 (*e.g.*, an ESE listed in Table 11).

[000295] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping, such as for skipping one or more of exons 8, 23, 42, 44, 45, 46, 50, 52, 53, and 55) comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of a DMD transcript (e.g., one or more full or partial ESEs listed in Table 15 or Table 11). In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 402-436 and 2043-2238. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 402-436 and 2043-2238.

Table 15. Exonic splicing enhancers within exon 51 of DMD

ESE #	Ref. start position*	SEQ ID NO:	ESE Motif Sequence	Name
51-1	1565421	402	CTACTCA	Dp427m_51:SRSF5
51-2	1565426	403	CAGACTG	Dp427m_51:SRSF1 (IgM-BRCA1)
51-3	1565428	404	GACTGTTA	Dp427m_51:SRSF2
51-4	1565433	405	TTACTCT	Dp427m_51:SRSF5
51-5	1565435	406	ACTCTGG	Dp427m_51:SRSF5
51-6	1565436	407	CTCTGGT	Dp427m_51:SRSF1 (IgM-BRCA1)
51-7	1565442	408	TGACACA	Dp427m_51:SRSF5
51-8	1565443	409	GACACAA	Dp427m_51:SRSF1
51-9	1565444	410	ACACAAC	Dp427m_51:SRSF5
51-10	1565448	411	AACCTGTG	Dp427m_51:SRSF2
51-11	1565450	412	CCTGTGG	Dp427m_51:SRSF5
51-12	1565451	413	CTGTGGT	Dp427m_51:SRSF1 (IgM-BRCA1)
51-13	1565455	414	GGTTACTA	Dp427m_51:SRSF2
51-14	1565457	415	TTACTAA	Dp427m_51:SRSF5
51-15	1565460	416	CTAAGGA	Dp427m_51:SRSF1 (IgM-BRCA1)
51-16	1565469	417	CTGCCAT	Dp427m_51:SRSF1 (IgM-BRCA1)
51-17	1565479	418	CAAACTA	Dp427m_51:SRSF1 (IgM-BRCA1)
51-18	1565508	419	TGGAGGT	Dp427m_51:SRSF1
51-19	1565512	420	GGTACCTG	Dp427m_51:SRSF2
51-20	1565528	421	GATTTCAA	Dp427m_51:SRSF2
51-21	1565530	422	TTTCAAC	Dp427m_51:SRSF5
51-22	1565532	423	TCAACCG	Dp427m_51:SRSF5
51-23	1565533	424	CAACCGG	Dp427m_51:SRSF1 (IgM-BRCA1)
51-24	1565544	425	GGACAGAA	Dp427m_51:SRSF2
51-25	1565556	426	CCGACTG	Dp427m_51:SRSF1 (IgM-BRCA1)
51-26	1565557	427	CGACTGG	Dp427m_51:SRSF5
51-27	1565565	428	TTTCTCTG	Dp427m_51:SRSF2
51-28	1565567	429	TCTCTGC	Dp427m_51:SRSF5
51-29	1565591	430	TCACAGA	Dp427m_51:SRSF5
51-30	1565592	431	CACAGA	Dp427m_51:SRSF6
51-31	1565593	432	ACAGAGG	Dp427m_51:SRSF5
51-32	1565594	433	CAGAGGG	Dp427m_51:SRSF1 (IgM-BRCA1)
51-33	1565615	434	CTTGAGG	Dp427m_51:SRSF5
51-34	1565617	435	TGAGGA	Dp427m_51:SRSF6
51-35	1565630	436	GAGATGA	Dp427m_51:SRSF1

*Ref. start position refers to the position of the first nucleotide of the ESE motif in nucleotides 5,001-2,225,382 of NCBI Reference Sequence NG_012232.1 (NG_012232, version 1). Nucleotides 5,001-2,225,382 of NCBI Reference Sequence NG_012232.1 (NG_012232, version 1) correspond to *Homo sapiens* dystrophin (DMD) gene on chromosome X.

Table 11. Exonic splicing enhancers within exons 8, 23, 43, 44, 45, 46, 50, 52, 53, and 55 of DMD

Exon	Ref. start position*	SEQ ID NO:	ESE Motif Sequence	Name
8	640346	2047	TCCATC	Dp427m_8:SRSF6
8	640358	2048	TACATC	Dp427m_8:SRSF6

Exon	Ref. start position*	SEQ ID NO:	ESE Motif Sequence	Name
8	640362	2049	TCACATC	Dp427m_8:SRSF5
8	640363	2050	CACATC	Dp427m_8:SRSF6
8	640367	2051	TCACTCT	Dp427m_8:SRSF5
8	640368	2052	CACTCTT	Dp427m_8:SRSF1 (IgM-BRCA1)
8	640373	2053	TTCCAAG	Dp427m_8:SRSF5
8	640383	2054	TGCCTC	Dp427m_8:SRSF6
8	640385	2055	CCTCAAC	Dp427m_8:SRSF5
8	640388	2056	CAACAAG	Dp427m_8:SRSF5
8	640434	2057	GGCCACCT	Dp427m_8:SRSF2
8	640439	2058	CCTAAAG	Dp427m_8:SRSF5
8	640447	2059	GACTAAAG	Dp427m_8:SRSF2
8	640469	2060	TTACATC	Dp427m_8:SRSF5
8	640470	2048	TACATC	Dp427m_8:SRSF6
8	640477	2061	TCAAATG	Dp427m_8:SRSF5
8	640490	2062	TCTCAAC	Dp427m_8:SRSF5
23	870903	2063	TTACAAA	Dp427m_23:SRSF5
23	870910	2064	GTTCTCTG	Dp427m_23:SRSF2
23	870912	429	TCTCTGC	Dp427m_23:SRSF5
23	870933	2065	GGCCTATA	Dp427m_23:SRSF2
23	870944	2066	TCTCAGC	Dp427m_23:SRSF5
23	870945	2067	CTCAGCA	Dp427m_23:SRSF1 (IgM-BRCA1)
23	870950	2068	CACCACTG	Dp427m_23:SRSF2
23	870952	2069	CCACTGT	Dp427m_23:SRSF5
23	870970	2070	CGAAGAA	Dp427m_23:SRSF1 (IgM-BRCA1)
23	870979	2071	CGCCCTC	Dp427m_23:SRSF1 (IgM-BRCA1)
23	870980	2072	GCCCTCTG	Dp427m_23:SRSF2
23	870993	2073	AGCCGGA	Dp427m_23:SRSF1 (IgM-BRCA1)
23	871014	2074	TTTGAAG	Dp427m_23:SRSF5
23	871028	2075	GGGACGC	Dp427m_23:SRSF1
23	871029	2076	GGACGCTG	Dp427m_23:SRSF2
23	871032	2077	CGCTGGA	Dp427m_23:SRSF1 (IgM-BRCA1)
23	871049	2078	CTCCCAG	Dp427m_23:SRSF1 (IgM-BRCA1)
23	871050	2079	TCCCAGC	Dp427m_23:SRSF5
23	871051	2080	CCCAGCT	Dp427m_23:SRSF1 (IgM-BRCA1)
23	871053	2081	CAGCTGG	Dp427m_23:SRSF1 (IgM-BRCA1)
23	871070	2082	TCAAAAG	Dp427m_23:SRSF5
23	871077	2083	CTAGAGG	Dp427m_23:SRSF5
23	871078	2084	TAGAGGA	Dp427m_23:SRSF1
23	871090	2085	TGAATA	Dp427m_23:SRSF6
23	871098	2086	CTCCGAA	Dp427m_23:SRSF1 (IgM-BRCA1)
43	1051912	2087	ATAAAAG	Dp427m_43:SRSF5
43	1051922	2088	GTCTACAA	Dp427m_43:SRSF2
43	1051924	2089	CTACAAC	Dp427m_43:SRSF5
43	1051929	2090	ACAAAGC	Dp427m_43:SRSF5
43	1051934	2091	GCTCAGG	Dp427m_43:SRSF5
43	1051935	2092	CTCAGGT	Dp427m_43:SRSF1 (IgM-BRCA1)
43	1051955	2093	TTCATA	Dp427m_43:SRSF6

Exon	Ref. start position*	SEQ ID NO:	ESE Motif Sequence	Name
43	1051956	2094	TCATAGC	Dp427m_43:SRSF5
43	1051967	2095	AGACAGC	Dp427m_43:SRSF5
43	1051971	2096	AGCAGC	Dp427m_43:SRSF6
43	1051986	2097	TGCAAC	Dp427m_43:SRSF6
43	1051991	2098	CGCCTGTG	Dp427m_43:SRSF2
43	1051993	412	CCTGTGG	Dp427m_43:SRSF5
43	1051994	2099	CTGTGGA	Dp427m_43:SRSF1 (IgM-BRCA1)
43	1051995	2100	TGTGGA	Dp427m_43:SRSF6
43	1052009	2101	AGCTACAG	Dp427m_43:SRSF2
43	1052011	2102	CTACAGG	Dp427m_43:SRSF5
43	1052012	2103	TACAGGA	Dp427m_43:SRSF1 (IgM-BRCA1)
43	1052022	2104	TCTCTCC	Dp427m_43:SRSF5
43	1052023	2105	CTCTCCCA	Dp427m_43:SRSF2
43	1052025	2078	CTCCCAG	Dp427m_43:SRSF1 (IgM-BRCA1)
43	1052026	2079	TCCCAGC	Dp427m_43:SRSF5
43	1052027	2080	CCCAGCT	Dp427m_43:SRSF1 (IgM-BRCA1)
43	1052035	2106	GATTTCCA	Dp427m_43:SRSF2
43	1052040	2107	CCAATGG	Dp427m_43:SRSF5
43	1052064	2108	GTACAAG	Dp427m_43:SRSF5
43	1052071	2109	GACCGACA	Dp427m_43:SRSF2
43	1052073	2110	CCGACAA	Dp427m_43:SRSF1 (IgM-BRCA1)
43	1052074	2111	CGACAAG	Dp427m_43:SRSF5
44	1122553	2112	TGACAGA	Dp427m_44:SRSF5
44	1122575	2113	CGGCGTT	Dp427m_44:SRSF1 (IgM-BRCA1)
44	1122607	2114	TCAGTGG	Dp427m_44:SRSF5
44	1122612	2115	GGCTAACA	Dp427m_44:SRSF2
44	1122617	2116	ACAGAAG	Dp427m_44:SRSF5
44	1122634	2117	TCTCAGA	Dp427m_44:SRSF5
44	1122635	2118	CTCAGAA	Dp427m_44:SRSF1 (IgM-BRCA1)
44	1122643	409	GACACAA	Dp427m_44:SRSF1
44	1122649	2119	AATTCCTG	Dp427m_44:SRSF2
44	1122654	2120	CTGAGAA	Dp427m_44:SRSF1 (IgM-BRCA1)
44	1122685	2121	GTATCTTA	Dp427m_44:SRSF2
45	1371096	2122	GAACTCCA	Dp427m_45:SRSF2
45	1371097	2123	AACTCCAG	Dp427m_45:SRSF2
45	1371099	2124	CTCCAGG	Dp427m_45:SRSF5
45	1371117	2125	CAGCGGC	Dp427m_45:SRSF1 (IgM-BRCA1)
45	1371118	2126	AGCGGC	Dp427m_45:SRSF6
45	1371133	2127	TCAGAAC	Dp427m_45:SRSF5
45	1371136	2128	GAACATTG	Dp427m_45:SRSF2
45	1371142	2129	TGAATGC	Dp427m_45:SRSF5
45	1371143	2130	GAATGCAA	Dp427m_45:SRSF2
45	1371146	2097	TGCAAC	Dp427m_45:SRSF6
45	1371148	2131	CAACTGG	Dp427m_45:SRSF5
45	1371151	2132	CTGGGGA	Dp427m_45:SRSF1 (IgM-BRCA1)
45	1371165	2133	ATTCAGC	Dp427m_45:SRSF5
45	1371188	2134	TGCCAGTA	Dp427m_45:SRSF2

Exon	Ref. start position*	SEQ ID NO:	ESE Motif Sequence	Name
45	1371193	2135	GTATTCTA	Dp427m_45:SRSF2
45	1371198	2102	CTACAGG	Dp427m_45:SRSF5
45	1371199	2103	TACAGGA	Dp427m_45:SRSF1 (IgM-BRCA1)
45	1371220	2136	TGAATC	Dp427m_45:SRSF6
45	1371225	2137	CTGCGGT	Dp427m_45:SRSF1 (IgM-BRCA1)
45	1371226	2138	TGCGGT	Dp427m_45:SRSF6
45	1371228	2139	CGGTGGC	Dp427m_45:SRSF1 (IgM-BRCA1)
45	1371231	2140	TGGCAGG	Dp427m_45:SRSF5
45	1371232	2141	GGCAGGA	Dp427m_45:SRSF1 (IgM-BRCA1)
45	1371235	2142	AGGAGGT	Dp427m_45:SRSF1
45	1371239	2143	GGTCTGCA	Dp427m_45:SRSF2
45	1371240	2144	GTCTGCAA	Dp427m_45:SRSF2
45	1371249	2145	CAGCTGT	Dp427m_45:SRSF1 (IgM-BRCA1)
45	1371256	2146	CAGACAG	Dp427m_45:SRSF1 (IgM-BRCA1)
46	1407384	2147	CTAGAAG	Dp427m_46:SRSF5
46	1407392	2148	ACAAAAG	Dp427m_46:SRSF5
46	1407438	2149	GTTTTATG	Dp427m_46:SRSF2
46	1407440	2150	TTTATGG	Dp427m_46:SRSF5
46	1407445	2151	GGTTGGAG	Dp427m_46:SRSF2
46	1407448	2152	TGGAGGA	Dp427m_46:SRSF1 (IgM-BRCA1)
46	1407472	2153	GTATCCCA	Dp427m_46:SRSF2
46	1407476	2154	CCCACTT	Dp427m_46:SRSF1 (IgM-BRCA1)
46	1407477	2155	CCACTTG	Dp427m_46:SRSF5
46	1407478	2156	CACTTGA	Dp427m_46:SRSF1 (IgM-BRCA1)
46	1407496	2096	AGCAGC	Dp427m_46:SRSF6
46	1407504	2157	CTAAAAG	Dp427m_46:SRSF5
46	1407524	2158	AGTCAAG	Dp427m_46:SRSF5
50	1519533	2159	TTAGAAG	Dp427m_50:SRSF5
50	1519539	2160	GATCTGAG	Dp427m_50:SRSF2
50	1519541	2161	TCTGAGC	Dp427m_50:SRSF5
50	1519542	2162	CTGAGCT	Dp427m_50:SRSF1 (IgM-BRCA1)
50	1519544	2163	GAGCTCTG	Dp427m_50:SRSF2
50	1519549	2164	CTGAGTG	Dp427m_50:SRSF1 (IgM-BRCA1)
50	1519550	2165	TGAGTGG	Dp427m_50:SRSF5
50	1519572	2166	TTACTTC	Dp427m_50:SRSF5
50	1519573	2167	TACTTC	Dp427m_50:SRSF6
50	1519575	2168	CTTCAAG	Dp427m_50:SRSF5
50	1519584	2169	CTGAGGG	Dp427m_50:SRSF1 (IgM-BRCA1)
50	1519594	2096	AGCAGC	Dp427m_50:SRSF6
50	1519596	2170	CAGCCTG	Dp427m_50:SRSF1 (IgM-BRCA1)
50	1519600	2171	CTGACCT	Dp427m_50:SRSF1 (IgM-BRCA1)
50	1519607	2172	AGCTCCTG	Dp427m_50:SRSF2
50	1519609	2173	CTCCTGG	Dp427m_50:SRSF5
50	1519617	2174	CTGACCA	Dp427m_50:SRSF1 (IgM-BRCA1)
50	1519619	2175	GACCACTA	Dp427m_50:SRSF2
50	1519621	2176	CCACTAT	Dp427m_50:SRSF5
50	1519624	2177	CTATTGG	Dp427m_50:SRSF5

Exon	Ref. start position*	SEQ ID NO:	ESE Motif Sequence	Name
52	1609869	2178	TGCAGG	Dp427m_52:SRSF6
52	1609880	2179	GAACAGAG	Dp427m_52:SRSF2
52	1609882	432	ACAGAGG	Dp427m_52:SRSF5
52	1609883	2180	CAGAGGC	Dp427m_52:SRSF1 (IgM-BRCA1)
52	1609887	2181	GGCGTC	Dp427m_52:SRSF6
52	1609889	2182	CGTCCCCA	Dp427m_52:SRSF2
52	1609890	2183	GTCCCCAG	Dp427m_52:SRSF2
52	1609892	2184	CCCCAGT	Dp427m_52:SRSF1 (IgM-BRCA1)
52	1609893	2185	CCCAGTT	Dp427m_52:SRSF1 (IgM-BRCA1)
52	1609911	2186	TTACCGC	Dp427m_52:SRSF5
52	1609912	2187	TACCGCTG	Dp427m_52:SRSF2
52	1609917	2188	CTGCCCA	Dp427m_52:SRSF1 (IgM-BRCA1)
52	1609939	2189	GACCAGCA	Dp427m_52:SRSF2
52	1609954	2190	GGCTAGAA	Dp427m_52:SRSF2
52	1609969	2191	TACGGA	Dp427m_52:SRSF6
52	1609972	2192	GGATCGAA	Dp427m_52:SRSF2
53	1660030	2193	GAATTCAG	Dp427m_53:SRSF2
53	1660040	2114	TCAGTGG	Dp427m_53:SRSF5
53	1660041	2194	CAGTGGG	Dp427m_53:SRSF1 (IgM-BRCA1)
53	1660053	2108	GTACAAG	Dp427m_53:SRSF5
53	1660067	2127	TCAGAAC	Dp427m_53:SRSF5
53	1660071	2195	AACCGGA	Dp427m_53:SRSF1
53	1660074	2196	CGGAGGC	Dp427m_53:SRSF1 (IgM-BRCA1)
53	1660098	2197	TTAAAGG	Dp427m_53:SRSF5
53	1660103	2198	GGATTCAA	Dp427m_53:SRSF2
53	1660112	2199	ACAATGG	Dp427m_53:SRSF5
53	1660117	2200	GGCTGGAA	Dp427m_53:SRSF2
53	1660126	416	CTAAGGA	Dp427m_53:SRSF1 (IgM-BRCA1)
53	1660138	2201	CTGAGCA	Dp427m_53:SRSF1 (IgM-BRCA1)
53	1660141	2202	AGCAGGT	Dp427m_53:SRSF1 (IgM-BRCA1)
53	1660147	2203	TCTTAGG	Dp427m_53:SRSF5
53	1660148	2204	CTTAGGA	Dp427m_53:SRSF1 (IgM-BRCA1)
53	1660152	2205	GGACAGG	Dp427m_53:SRSF5
53	1660153	2206	GACAGGC	Dp427m_53:SRSF1
53	1660157	2207	GGCCAGAG	Dp427m_53:SRSF2
53	1660159	2208	CCAGAGC	Dp427m_53:SRSF5
53	1660172	2209	TGAGTC	Dp427m_53:SRSF6
53	1660183	2210	AGGAGGG	Dp427m_53:SRSF1
53	1660188	2211	GGTCCCTA	Dp427m_53:SRSF2
53	1660197	2212	ACAGTAG	Dp427m_53:SRSF5
53	1660211	2213	CCAAAAG	Dp427m_53:SRSF5
53	1660222	430	TCACAGA	Dp427m_53:SRSF5
53	1660223	431	CACAGA	Dp427m_53:SRSF6
55	1711758	2214	CGAGAGG	Dp427m_55:SRSF5
55	1711763	2215	GGCTGCTT	Dp427m_55:SRSF2
55	1711786	2216	GATTACTG	Dp427m_55:SRSF2
55	1711788	2217	TTACTGC	Dp427m_55:SRSF5

Exon	Ref. start position*	SEQ ID NO:	ESE Motif Sequence	Name
55	1711792	2097	TGCAAC	Dp427m_55:SRSF6
55	1711802	2218	CCCCCTG	Dp427m_55:SRSF1 (IgM-BRCA1)
55	1711803	2219	CCCCTGG	Dp427m_55:SRSF5
55	1711804	2220	CCCTGGA	Dp427m_55:SRSF1 (IgM-BRCA1)
55	1711820	2221	GTTTCTTG	Dp427m_55:SRSF2
55	1711821	2222	TTTCTTG	Dp427m_55:SRSF5
55	1711826	2223	TGCCTGG	Dp427m_55:SRSF5
55	1711831	2224	GGCTTACA	Dp427m_55:SRSF2
55	1711834	2225	TTACAGA	Dp427m_55:SRSF5
55	1711835	2226	TACAGA	Dp427m_55:SRSF6
55	1711836	2116	ACAGAAG	Dp427m_55:SRSF5
55	1711852	2227	CTGCCAA	Dp427m_55:SRSF1 (IgM-BRCA1)
55	1711853	2228	TGCCAATG	Dp427m_55:SRSF2
55	1711860	2229	GTCCTACA	Dp427m_55:SRSF2
55	1711863	2102	CTACAGG	Dp427m_55:SRSF5
55	1711864	2103	TACAGGA	Dp427m_55:SRSF1 (IgM-BRCA1)
55	1711868	2230	GGATGCTA	Dp427m_55:SRSF2
55	1711873	2231	CTACCCG	Dp427m_55:SRSF5
55	1711874	2232	TACCCGTA	Dp427m_55:SRSF2
55	1711888	2233	GGCTCCTA	Dp427m_55:SRSF2
55	1711893	2147	CTAGAAG	Dp427m_55:SRSF5
55	1711898	2234	AGACTCC	Dp427m_55:SRSF5
55	1711899	2235	GACTCCAA	Dp427m_55:SRSF2
55	1711901	2236	CTCCAAG	Dp427m_55:SRSF5
55	1711903	2237	CCAAGGG	Dp427m_55:SRSF1 (IgM-BRCA1)
55	1711920	2238	CTGATGA	Dp427m_55:SRSF1 (IgM-BRCA1)
55	1711928	2199	ACAATGG	Dp427m_55:SRSF5

*Ref. start position refers to the position of the first nucleotide of the ESE motif in nucleotides 5,001-2,225,382 of NCBI Reference Sequence NG_012232.1 (NG_012232, version 1). Nucleotides 5,001-2,225,382 of NCBI Reference Sequence NG_012232.1 (NG_012232, version 1) correspond to *Homo sapiens* dystrophin (DMD) gene on chromosome X.

[000296] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 8. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 8. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 2047-2062. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2047-2062.

[000297] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14,

15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 8. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 2047-2062.

[000298] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2047-2062. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2047-2062. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2047-2062. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2047-2062.

[000299] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 23. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 23. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 429 and 2063-2086. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 429 and 2063-2086.

[000300] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 23. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14,

15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 429 and 2063-2086.

[000301] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 429 and 2063-2086. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 429 and 2063-2086. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 429 and 2063-2086. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 429 and 2063-2086.

[000302] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 43. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 43. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 412, 2078-2080, and 2087-2111. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 412, 2078-2080, and 2087-2111.

[000303] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 43. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 412, 2078-2080, and 2087-2111.

[000304] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 412, 2078-2080, and 2087-2111. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 412, 2078-2080, and 2087-2111. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 412, 2078-2080, and 2087-2111. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 412, 2078-2080, and 2087-2111.

[000305] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 44. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 44. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 409 and 2112-2121. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 409 and 2112-2121.

[000306] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 44. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 409 and 2112-2121.

[000307] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE

as set forth in any one of SEQ ID NOs: 409 and 2112-2121. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 409 and 2112-2121. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 409 and 2112-2121.In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 409 and 2112-2121.

[000308] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 45. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 45. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 2097, 2102, 2103, and 2122-2146. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, and 2122-2146.

[000309] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 45. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 2097, 2102, 2103, and 2122-2146.

[000310] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, and 2122-2146. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30

(e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, and 2122-2146. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, and 2122-2146. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, and 2122-2146.

[000311] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 46. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 46. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 2096 and 2147-2158. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2147-2158.

[000312] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 46. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 2096 and 2147-2158.

[000313] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2147-2158. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence

comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2147-2158. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2147-2158. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2147-2158.

[000314] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 50. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 50. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 2096 and 2160-2177. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2160-2177.

[000315] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 50. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 2096 and 2160-2177.

[000316] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2160-2177. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2160-2177. In some embodiments, an oligonucleotide useful for

targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2160-2177. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2096 and 2160-2177.

[000317] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 51. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 51. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 402-436. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 402-436. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, 8) consecutive nucleotides of an ESE as set forth in SEQ ID NO: 419.

[000318] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 51. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 402-436. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, or 14) nucleotides of ESEs as set forth in SEQ ID NO: 418 and SEQ ID NO: 419.

[000319] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 402-436. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4

(e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 402-436. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 402-436. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 402-436.

[000320] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in SEQ ID NO: 419. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in SEQ ID NO: 419.

[000321] In some embodiments, the oligonucleotide is 20-30 (e.g., 20, 25, 30) nucleotides in length and comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, or 14) nucleotides of ESEs as set forth in SEQ ID NO: 418 and SEQ ID NO: 419. In some embodiments, the oligonucleotide is 30 nucleotides in length and comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, or 14) nucleotides of ESEs as set forth in SEQ ID NO: 418 and SEQ ID NO: 419.

[000322] Non-limiting examples of oligonucleotides that are useful for DMD exon 51 skipping and their target sequences are provided in SEQ ID NOs: 437-1241 and SEQ ID NOs: 1242-2046, respectively. In some embodiments, the oligonucleotide is 20-30 nucleotides in length and comprises a region of complementarity to a target sequence comprising at least 20 consecutive nucleotides of any one of SEQ ID NOs: 1242-2046. In some embodiments, the oligonucleotide is 20-30 nucleotides in length and comprises at least 20 consecutive nucleotides of any one of SEQ ID NOs: 437-1241. In some embodiments, the oligonucleotide comprises the nucleotide sequence of any one of SEQ ID NOs: 437-1241. In some embodiments, the oligonucleotide is at least 30 nucleotides (e.g., 30, 31, 32, 33, 34, or 35) in length and comprises the nucleotide sequence of any one of SEQ ID NOs: 437-1241.

[000323] In some embodiments, the oligonucleotide is 20-30 nucleotides in length and comprises a region of complementarity to a target sequence comprising at least 20 consecutive

nucleotides of any one of SEQ ID NOs: 1548, 1550, 1551, 1552, 1555, 1558, 1559, 1562, 1565, 1569, 1577, 1583, 1589, 1595, 1600, 1606, 1610, 1614, 1621, 1626, 1629, 1632, 1637, 1640, 1643, 1646, 1650, 1655, 1658, and 1662. In some embodiments, the oligonucleotide is 20-30 nucleotides in length and comprises at least 20 consecutive nucleotides of any one of SEQ ID NO: 743, 745, 746, 747, 750, 753, 754, 757, 760, 764, 772, 778, 784, 790, 795, 801, 805, 809, 816, 821, 824, 827, 832, 835, 838, 841, 845, 850, 853, and 857. In some embodiments, the oligonucleotide comprises the nucleotide sequence of any one of SEQ ID NOs: 743, 745, 746, 747, 750, 753, 754, 757, 760, 764, 772, 778, 784, 790, 795, 801, 805, 809, 816, 821, 824, 827, 832, 835, 838, 841, 845, 850, 853, and 857. In some embodiments, the oligonucleotide is 30 nucleotides in length and comprises the nucleotide sequence of any one of SEQ ID NOs: 743, 745, 746, 747, 750, 753, 754, 757, 760, 764, 772, 778, 784, 790, 795, 801, 805, 809, 816, 821, 824, 827, 832, 835, 838, 841, 845, 850, 853, and 857.

[000324] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 52. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 52. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 432 and 2178-2192. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 432 and 2178-2192.

[000325] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 52. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 432 and 2178-2192.

[000326] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 432 and 2178-2192. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence

comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 432 and 2178-2192. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 432 and 2178-2192. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 432 and 2178-2192.

[000327] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 53. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 53. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 416, 430, 431, 2108, 2114, 2127, and 2193-2213. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 416, 430, 431, 2108, 2114, 2127, and 2193-2213.

[000328] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 53. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 416, 430, 431, 2108, 2114, 2127, and 2193-2213.

[000329] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 416, 430, 431, 2108, 2114, 2127, and 2193-2213. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 416, 430, 431, 2108, 2114, 2127, and 2193-2213. In

some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 416, 430, 431, 2108, 2114, 2127, and 2193-2213. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 416, 430, 431, 2108, 2114, 2127, and 2193-2213.

[000330] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs of DMD exon 55. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE of DMD exon 55. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 2097, 2102, 2103, 2116, 2147, 2199, and 2214-2238. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, 2116, 2147, 2199, and 2214-2238.

[000331] In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) of DMD exon 55. In some embodiments, the oligonucleotide comprises a region of complementarity to a target sequence comprising at least 6 (e.g., 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) nucleotides of one or more ESEs (e.g., 2, 3, 4, or more adjacent ESEs) as set forth in SEQ ID NOs: 2097, 2102, 2103, 2116, 2147, 2199, and 2214-2238.

[000332] In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 18-35 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, 2116, 2147, 2199, and 2214-2238. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 20-30 (e.g., 20, 25, 30) nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, 2116, 2147, 2199, and 2214-2238. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is

20 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, 2116, 2147, 2199, and 2214-2238. In some embodiments, an oligonucleotide useful for targeting DMD (*e.g.*, for exon skipping) is 30 nucleotides in length, and comprises a region of complementarity to a target sequence comprising at least 4 (e.g., 4, 5, 6, 7, or 8) consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 2097, 2102, 2103, 2116, 2147, 2199, and 2214-2238.

[000333] In some embodiments, any one of the oligonucleotides useful for targeting DMD (*e.g.*, for exon skipping) is a phosphorodiamidate morpholino oligomer (PMO).

[000334] Additional examples of oligonucleotides targeting DMD (e.g., for exon skipping) are provided in U.S. Patent Application Publication 2013-072541, published March 21, 2013, entitled "ADENO-ASSOCIATED VIRAL VECTOR FOR EXON SKIPPING IN A GENE ENCODING A DISPENSIBLE-DOMAIN PROTEIN"; U.S. Patent Application Publication 2015-191725, published July 9, 2015, entitled "OLIGONUCLEOTIDE FOR THE TREATMENT OF MUSCULAR DYSTROPHY PATIENTS"; U.S. Patent Application Publication 2015-196670, published July 16, 2015, entitled "COMPOSITIONS AND METHODS FOR DUCHENNE MUSCULAR DYSTROPHY GENE THERAPY"; U.S. Patent Application Publication 2017-349905, published December 7, 2017, entitled "GENOME EDITING WITH SPLIT CAS9 EXPRESSED FROM TWO VECTORS"; U.S. Patent Application Publication 2018-028554, published February 1, 2018, entitled "OLIGOMERS HAVING BICYCLIC SCAFFOLD MOEITIES"; U.S. Patent Application Publication 2018-171333, published June 21, 2018, entitled "ANTISENSE MOLECULES AND METHODS FOR TREATING PATHOLOGIES"; U.S. Patent Application Publication 2018-179538, published June 28, 2018, entitled "ANTISENSE NUCLEIC ACIDS"; U.S. Patent Application Publication 2018-265859, published September 20, 2018, entitled "MODIFICATION OF THE DYSTROPHIN GENE AND USES THEREOF"; U.S. Patent Application Publication 2018-369400, published December 27, 2018, entitled "NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND METHODS OF INDUCING EXON SKIPPING"; U.S. Patent Application Publication 2019-000986, published January 3, 2019, entitled "NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND METHODS OF INDUCING EXON SKIPPING"; U.S. Patent Application Publication 2019-008986, published January 10, 2019, entitled "OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF"; U.S. Patent Application Publication 2019-112604, published April 18, 2019, entitled "METHODS AND MEANS FOR EFFICIENT SKIPPING OF EXON 45 IN DUCHENNE MUSCULAR

DYSTROPHY PRE-MRNA"; U.S. Patent Application Publication 2019-119679, published April 25, 2019, entitled "METHODS AND MEANS FOR EFFICIENT SKIPPING OF EXON 45 IN DUCHENNE MUSCULAR DYSTROPHY PRE-MRNA"; U.S. Patent Application Publication 2019-127733, published May 2, 2019, entitled "OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF"; U.S. Patent Application Publication 2019-151476, published May 23, 2019, entitled "THERAPEUTIC APPLICATIONS OF CPF1-BASED GENOME EDITING"; U.S. Patent Application Publication 2019-177723, published June 13, 2019, entitled "COMPOSITIONS AND METHODS FOR TREATING DUCHENNE MUSCULAR DYSTROPHY AND RELATED DISORDERS"; U.S. Patent Application Publication 2019-177725, published June 13, 2019, entitled "METHODS AND MEANS FOR EFFICIENT SKIPPING OF EXON 45 IN DUCHENNE MUSCULAR DYSTROPHY PRE-MRNA"; U.S. Patent Application Publication 2019-209604, published July 11, 2019, entitled "OLIGONUCLEOTIDES, COMPOSITIONS AND METHODS THEREOF"; U.S. Patent Application Publication 2019-249173, published August 15, 2019, entitled "METHODS AND COMPOSITIONS OF BIOLOGICALLY ACTIVE AGENTS"; U.S. Patent Application Publication 2019-270994, published September 5, 2019, entitled "ANTISENSE MOLECULES AND METHODS FOR TREATING PATHOLOGIES"; U.S. Patent Application Publication 2019-284556, published September 19, 2019, entitled "MULTIPLE EXON SKIPPING COMPOSITIONS FOR DMD"; U.S. Patent Application Publication 2019-323010, published October 24, 2019, entitled "ANTISENSE OLIGONUCLEOTIDES FOR INDUCING EXON SKIPPING AND METHODS OF USE THEREOF"; U.S. Patent Application Publication 2019-330626, published October 31, 2019, entitled "COMPOUNDS AND METHODS FOR USE IN DYSTROPHIN TRANSCRIPT"; U.S. Patent Application Publication 2019-338311, published November 7, 2019, entitled "OPTIMIZED STRATEGY FOR EXON SKIPPING MODIFICATIONS USING CRISPR/CAS9 WITH TRIPLE GUIDE SEQUENCES"; U.S. Patent Application Publication 2019-359982, published November 28, 2019, entitled "COMPOSITIONS FOR TREATING MUSCULAR DYSTROPHY"; U.S. Patent Application Publication 2019-364862, published December 5, 2019, entitled "DMD REPORTER MODELS CONTAINING HUMANIZED DUCHENNE MUSCULAR DYSTROPHY MUTATIONS"; U.S. Patent Application Publication 2019-390197, published December 26, 2019, entitled "OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF"; U.S. Patent Application Publication 2020-040337, published February 6, 2020, entitled "COMPOSITIONS FOR TREATING MUSCULAR DYSTROPHY"; U.S. Patent No. 10,287,586, issued May 14, 2019, entitled "ANTISENSE MOLECULES AND METHODS

FOR TREATING PATHOLOGIES"; U.S. Patent No. 10,337,003, issued July 2, 2019, entitled "COMPOSITIONS FOR TREATING MUSCULAR DYSTROPHY"; U.S. Patent No. 10,364,431, issued July 30, 2019, entitled "COMPOSITIONS FOR TREATING MUSCULAR DYSTROPHY"; U.S. Patent No. 10,450,568, issued October 22, 2019, entitled "OLIGONUCLEOTIDE COMPOSITIONS AND METHODS THEREOF"; U.S. Patent No. 10,487,106, issued November 26, 2019, entitled "ANTISENSE NUCLEIC ACIDS"; U.S. Patent No. 10,533,171, issued January 14, 2020, entitled "OLIGONUCLEOTIDE COMPRISING AN INOSINE FOR TREATING DMD"; U.S. Patent No. 10,704,060, issued July 7, 2020, entitled "RNA-GUIDED GENE EDITING AND GENE REGULATION"; U.S. Patent No. 10,752,898, issued August 25, 2020, entitled "EFFECTIVE GENE THERAPY TOOLS FOR DYSTROPHIN EXON 53 SKIPPING"; U.S. Patent No. 10,876,114, issued December 29, 2020, entitled "METHODS AND MEANS FOR EFFICIENT SKIPPING OF AT LEAST ONE OF THE FOLLOWING EXONS OF THE HUMAN DUCHENNE MUSCULAR DYSTROPHY GENE: 43, 46, 50-53"; U.S. Patent No. 6,100,099, issued August 8, 2000, entitled "TEST STRIP HAVING A DIAGONAL ARRAY OF CAPTURE SPOTS"; U.S. Patent No. 6,210,898, issued April 3, 2001, entitled "METHOD OF PERFORMING IMMUNOCHROMATOGRAPHY"; U.S. Patent No. 7,973,015, issued July 5, 2011, entitled "INDUCTION OF EXON SKIPPING IN EUKARYOTIC CELLS"; U.S. Patent No. 8,039,608, issued October 18, 2011, entitled "BIOINFORMATICALLY DETECTABLE GROUP OF NOVEL REGULATORY GENES AND USES THEREOF"; U.S. Patent No. 8,361,979, issued January 29, 2013, entitled "MEANS AND METHOD FOR INDUCING EXON-SKIPPING"; U.S. Patent No. 8,802,437, issued August 12, 2014, entitled "MEGANUCLEASE REAGENTS OF USES THEREOF FOR TREATING GENETIC DISEASES CAUSED BY FRAME SHIFT/NON SENSE MUTATIONS"; U.S. Patent No. 8,865,883, issued October 21, 2014, entitled "MULTIPLE EXON SKIPPING COMPOSITIONS FOR DMD"; U.S. Patent No. 9,657,049, issued May 23, 2017, entitled "ENA NUCLEIC ACID PHARMACEUTICALS CAPABLE OF MODIFYING SPLICING OF MRNA PRECURSORS"; U.S. Patent No. 9,657,050, issued May 23, 2017, entitled "ENA NUCLEIC ACID PHARMACEUTICALS CAPABLE OF MODIFYING SPLICING OF MRNA PRECURSORS"; U.S. Patent No. 9,988,629, issued June 5, 2018, entitled "ANTISENSE NUCLEIC ACIDS"; International Patent Publication WO 2011/078797 A2, published June 30, 2011, entitled "ANTISENSE OLIGONUCLEOTIDES AND USES THREREOF"; International Patent Publication WO 2011/154427 A1, published December 15, 2011, entitled "MODIFIED SNRNAS FOR USE IN THERAPY"; International Patent

Publication WO 2018/007475 A1, published January 11, 2018, entitled "PRE-MRNA SPLICE SWITCHING OR MODULATING OLIGONUCLEOTIDES COMPRISING BICYCLIC SCAFFOLD MOIETIES, WITH IMPROVED CHARACTERISTICS FOR THE TREATMENT OF GENETIC DISORDERS"; International Patent Publication WO 2018/014042 A1, published January 18, 2018, entitled "COMPOUNDS AND METHODS FOR MODULATION OF DYSTROPHIN TRANSCRIPT"; International Patent Publication WO 2018/017754 A1, published January 25, 2018, entitled "THERAPEUTIC APPLICATIONS OF CPF1-BASED GENOME EDITING"; International Patent Publication WO 2018/107003 A1, published June 14, 2018, entitled "DMD REPORTER MODELS CONTAINING HUMANIZED DUSCHENE MUSCULAR DYSTROPHY MUTATIONS"; International Patent Publication WO 2018/129296 A1, published July 12, 2018, entitled "OPTIMIZED STRATEGY FOR EXON SKIPPING MODIFICATIONS USING CRISPR/CAS9 WITH TRIPLE GUIDE SEQUENCES"; International Patent Publication WO 2019/014772 A1, published January 24, 2019, entitled "ANTISENSE OLIGONUCLEOTIDES THAT BIND TO EXON 51 OF HUMAN DYSTROPHIN PRE-MRNA"; International Patent Publication WO 2019/059973 A1, published March 28, 2019, entitled "EXON SKIPPING OLIGOMER CONJUGATES FOR MUSCULAR DYSTROPHY"; International Patent Publication WO 2019/060775 A1, published March 28, 2019, entitled "NUCLEIC ACID-POLYPEPTIDE COMPOSITIONS AND METHODS OF INDUCING EXON SKIPPING"; International Patent Publication WO 2019/067975 A1, published April 4, 2019, entitled "COMBINATION THERAPIES FOR TREATING MUSCULAR DYSTROPHY"; International Patent Publication WO 2019/092507 A2, published May 16, 2019, entitled "CRISPR/CAS SYSTEMS FOR TREATMENT OF DMD"; International Patent Publication WO 2019/136216 A1, published July 11, 2019, entitled "THERAPEUTIC CRISPR/CAS9 COMPOSITIONS AND METHODS OF USE"; International Patent Publication WO 2019/152609 A1, published August 8, 2019, entitled "COMPOSITIONS AND METHODS FOR CORRECTING DYSTROPHIN MUTATIONS IN HUMAN CARDIOMYOCYTES"; International Patent Publication WO 2019/200185 A1, published October 17, 2019, entitled "OLIGONUCLEOTIDE COMPOSITIONS AND METHODS OF USE THEREOF"; International Patent Publication WO 2019/215333 A1, published November 14, 2019, entitled "OLIGONUCLEOTIDES CONJUGATES COMPRISING 7'-5'-ALPHA-ANOMERIC-BICYCLIC SUGAR NUCLEOSIDES"; International Patent Publication WO 2019/241385 A2, published December 19, 2019, entitled "EXON SKIPPING OLIGOMERS FOR MUSCULAR DYSTROPY"; International Patent Publication WO 2019/246480 A1, published December 26,

2019, entitled "CORRECTION OF DYSTROPHIN EXON 43, EXON 45, OR EXON 52 DELETIONS IN DUCHENNE MUSCULAR DYSTROPHY"; International Patent Publication WO 2020/028832 A1, published February 6, 2020, entitled "MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING DYSTROPHINOPATHIES"; International Patent Publication WO 2018/091544 A1, published May 24, 2018, entitled "SUBSTANCES FOR TARGETING VARIOUS SELECTED ORGANS OR TISSUES"; International Patent Publication WO 2018/098480 A1, published May 31, 2018, entitled "PREVENTION OF MUSCULAR DYSTROPHY BY CRISPR/CPF1-MEDIATED GENE EDITING"; International Patent Publication WO 1993/020227 A1, published October 14, 1993, entitled "METHOD OF MULTIPLEX LIGASE CHAIN REACTION"; International Patent Publication WO 2013/100190 A1, published July 4, 2013, entitled "ANTISENSE NUCLEIC ACID"; International Patent Publication WO 2013/163628 A2, published October 31, 2013, entitled "GENETIC CORRECTION OF MUTATED GENES"; International Patent Publication WO 2007/135105 A1, published November 29, 2007, entitled "MEANS AND METHOD FOR INDUCING EXON-SKIPPING"; International Patent Publication WO 2011/150408 A2, published December 1, 2011, entitled "OLIGONUCLEOTIDE ANALOGUES HAVING MODIFIED INTERSUBUNIT LINKAGES AND/OR TERMINAL GROUPS"; International Patent Publication WO 2012/029986 A1 , published March 8, 2012, entitled "ANTISENSE NUCLEIC ACID"; the contents of each of which are incorporated herein in their entireties.

[000335] Examples of oligonucleotides for promoting DMD gene editing include International Patent Publication WO2018053632A1, published March 29, 2018, entitled "METHODS OF MODIFYING THE DYSTROPHIN GENE AND RESTORING DYSTROPHIN EXPRESSION AND USES THEREOF"; International Patent Publication WO2017049407A1, published March 30, 2017, entitled "MODIFICATION OF THE DYSTROPHIN GENE AND USES THEREOF"; International Patent Publication WO2016161380A1, published October 6, 2016, entitled "CRISPR/CAS-RELATED METHODS AND COMPOSITIONS FOR TREATING DUCHENNE MUSCULAR DYSTROPHY AND BECKER MUSCULAR DYSTROPHY"; International Patent Publication WO2017095967, published June 8, 2017, entitled "THERAPEUTIC TARGETS FOR THE CORRECTION OF THE HUMAN DYSTROPHIN GENE BY GENE EDITING AND METHODS OF USE"; International Patent Publication WO2017072590A1, published May 4, 2017, entitled "MATERIALS AND METHODS FOR TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY"; International Patent Publication

WO2018098480A1, published May 31, 2018, entitled "PREVENTION OF MUSCULAR DYSTROPHY BY CRISPR/CPF1-MEDIATED GENE EDITING"; US Patent Application Publication US20170266320A1, published September 21, 2017, entitled "RNA-Guided Systems for In Vivo Gene Editing"; International Patent Publication WO2016025469A1, published February 18, 2016, entitled "PREVENTION OF MUSCULAR DYSTROPHY BY CRISPR/CAS9-MEDIATED GENE EDITING"; U.S. Patent Application Publication 2016/0201089, published July 14, 2016, entitled "RNA-GUIDED GENE EDITING AND GENE REGULATION"; and U.S. Patent Application Publication 2013/0145487, published June 6, 2013, entitled "MEGANUCLEASE VARIANTS CLEAVING A DNA TARGET SEQUENCE FROM THE DYSTROPHN GENE AND USES THEREOF", the contents of each of which are incorporated herein in their entireties. In some embodiments, an oligonucleotide may have a region of complementarity to DMD gene sequences of multiple species, e.g., selected from human, mouse and non-human species.

[000336] In some embodiments, the oligonucleotide may have region of complementarity to a mutant DMD allele, for example, a DMD allele with at least one mutation in any of exons 1-79 of DMD in humans that leads to a frameshift and improper RNA splicing/processing.

[000337] In some embodiments, the oligonucleotide may target lncRNA or mRNA, *e.g.*, for degradation. In some embodiments, the oligonucleotide may target, *e.g.*, for degradation, a nucleic acid encoding a protein involved in a mismatch repair pathway, *e.g.*, MSH2, MutLalpha, MutSbeta, MutLalpha. Non-limiting examples of proteins involved in mismatch repair pathways, for which mRNAs encoding such proteins may be targeted by oligonucleotides described herein, are described in Iyer, R.R. et al., "*DNA triplet repeat expansion and mismatch repair*" Annu Rev Biochem. 2015;84:199-226.; and Schmidt M.H. and Pearson C.E., "Disease-associated repeat instability and mismatch repair" DNA Repair (Amst). 2016 Feb;38:117-26.

[000338] In some embodiments, any one of the oligonucleotides can be in salt form, e.g., as sodium, potassium, or magnesium salts.

[000339] In some embodiments, the 5' or 3' nucleoside (e.g., terminal nucleoside) of any one of the oligonucleotides described herein is conjugated to an amine group, optionally via a spacer. In some embodiments, the spacer comprises an aliphatic moiety. In some embodiments, the spacer comprises a polyethylene glycol moiety. In some embodiments, a phosphodiester linkage is present between the spacer and the 5' or 3' nucleoside of the oligonucleotide. In some embodiments, the 5' or 3' nucleoside (e.g., terminal nucleoside) of any of the oligonucleotides described herein is conjugated to a spacer that is a substituted or

unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclylene, substituted or unsubstituted heterocyclylene, substituted or unsubstituted arylene, substituted or unsubstituted heteroarylene, -O-, -N(R^A)-, -S-, -C(=O)-, -C(=O)O-, -C(=O)NR^A-, -NR^AC(=O)-, -NR^AC(=O)R^A-, -C(=O)R^A-, -NR^AC(=O)O-, -NR^AC(=O)N(R^A)-, -OC(=O)-, -OC(=O)O-, -OC(=O)N(R^A)-, -$S(O)_2$NR^A-, -N$R^A$$S(O)_2$-, or a combination thereof; each R^A is independently hydrogen or substituted or unsubstituted alkyl. In certain embodiments, the spacer is a substituted or unsubstituted alkylene, substituted or unsubstituted heterocyclylene, substituted or unsubstituted heteroarylene, -O-, -N(R^A)-, or -C(=O)N(R^A)$_2$, or a combination thereof.

[000340] In some embodiments, the 5' or 3' nucleoside of any one of the oligonucleotides described herein is conjugated to a compound of the formula -NH_2-$(CH_2)_n$-, wherein n is an integer from 1 to 12. In some embodiments, n is 6, 7, 8, 9, 10, 11, or 12. In some embodiments, a phosphodiester linkage is present between the compound of the formula NH_2-$(CH_2)_n$- and the 5' or 3' nucleoside of the oligonucleotide. In some embodiments, a compound of the formula NH_2-$(CH_2)_6$- is conjugated to the oligonucleotide via a reaction between 6-amino-1-hexanol (NH_2-$(CH_2)_6$-OH) and the 5' phosphate of the oligonucleotide.

[000341] In some embodiments, the oligonucleotide is conjugated to a targeting agent, e.g., a muscle targeting agent such as an anti-TfR antibody, e.g., via the amine group.

a. Oligonucleotide Size/Sequence

[000342] Oligonucleotides may be of a variety of different lengths, e.g., depending on the format. In some embodiments, an oligonucleotide is 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 75, or more nucleotides in length. In some embodiments, the oligonucleotide is 8 to 50 nucleotides in length, 8 to 40 nucleotides in length, 8 to 30 nucleotides in length, 10 to 15 nucleotides in length, 10 to 20 nucleotides in length, 15 to 25 nucleotides in length, 21 to 23 nucleotides in lengths, etc.

[000343] In some embodiments, a complementary nucleic acid sequence of an oligonucleotide for purposes of the present disclosure is specifically hybridizable or specific for the target nucleic acid when binding of the sequence to the target molecule (e.g., mRNA) interferes with the function of the target (e.g., mRNA) to cause a change of activity (e.g., inhibiting translation, altering splicing, exon skipping) or expression (e.g., degrading a target mRNA) and there is a sufficient degree of complementarity to avoid non-specific binding of the sequence to non-target sequences under conditions in which avoidance of non-specific binding is desired, e.g., under physiological conditions in the case of in vivo assays or

therapeutic treatment, and in the case of in vitro assays, under conditions in which the assays are performed under suitable conditions of stringency. Thus, in some embodiments, an oligonucleotide may be at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% complementary to the consecutive nucleotides of a target nucleic acid. In some embodiments a complementary nucleotide sequence need not be 100% complementary to that of its target to be specifically hybridizable or specific for a target nucleic acid.

[000344] In some embodiments, an oligonucleotide comprises region of complementarity to a target nucleic acid that is in the range of 8 to 15, 8 to 30, 8 to 40, or 10 to 50, or 5 to 50, or 5 to 40 nucleotides in length. In some embodiments, a region of complementarity of an oligonucleotide to a target nucleic acid is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length. In some embodiments, the region of complementarity is complementary with at least 8 consecutive nucleotides of a target nucleic acid. In some embodiments, an oligonucleotide may contain 1, 2 or 3 base mismatches compared to the portion of the consecutive nucleotides of target nucleic acid. In some embodiments the oligonucleotide may have up to 3 mismatches over 15 bases, or up to 2 mismatches over 10 bases.

[000345] In some embodiments, the oligonucleotide is complementary (e.g., at least 85% at least 90%, at least 95%, or 100%) to a target sequence of the any one of the oligonucleotides described herein (e.g., the oligonucleotides listed in Table 14). In some embodiments, the oligonucleotide is complementary (e.g., at least 85% at least 90%, at least 95%, or 100%) to a target sequence of the any one of the oligonucleotides provided by SEQ ID NO: 437-1241. In some embodiments, such target sequence is 100% complementary to an oligonucleotide listed in Table 14. In some embodiments, such target sequence is 100% complementary to an oligonucleotide provided by SEQ ID NO: 437-1241. In some embodiments, the oligonucleotide is complementary (e.g., at least 85% at least 90%, at least 95%, or 100%) to a target sequence provided herein (e.g., a target sequence of any one of the oligonucleotides listed in Table 14). In some embodiments, the oligonucleotide is complementary (e.g., at least 85% at least 90%, at least 95%, or 100%) to a target sequence of any one of the oligonucleotides provided by SEQ ID NO: 1242-2046.

[000346] In some embodiments, any one or more of the thymine bases (T's) in any one of the oligonucleotides provided herein (e.g., the oligonucleotides listed in Table 14) may optionally be uracil bases (U's), and/or any one or more of the U's in the oligonucleotides

provided herein may optionally be T's. In some embodiments, any one or more of the thymine bases (T's) in any one of the oligonucleotides provided by SEQ ID NOs: 437-1241 or in an oligonucleotide complementary to any one of SEQ ID NOs: 1242-2046 may optionally be uracil bases (U's), and/or any one or more of the U's in the oligonucleotides may optionally be T's.

b. Oligonucleotide Modifications:

[000347] The oligonucleotides described herein may be modified, e.g., comprise a modified sugar moiety, a modified internucleoside linkage, a modified nucleotide and/or (e.g., and) combinations thereof. In addition, in some embodiments, oligonucleotides may exhibit one or more of the following properties: do not mediate alternative splicing; are not immune stimulatory; are nuclease resistant; have improved cell uptake compared to unmodified oligonucleotides; are not toxic to cells or mammals; have improved endosomal exit internally in a cell; minimizes TLR stimulation; or avoid pattern recognition receptors. Any of the modified chemistries or formats of oligonucleotides described herein can be combined with each other. For example, one, two, three, four, five, or more different types of modifications can be included within the same oligonucleotide.

[000348] In some embodiments, certain nucleotide modifications may be used that make an oligonucleotide into which they are incorporated more resistant to nuclease digestion than the native oligodeoxynucleotide or oligoribonucleotide molecules; these modified oligonucleotides survive intact for a longer time than unmodified oligonucleotides. Specific examples of modified oligonucleotides include those comprising modified backbones, for example, modified internucleoside linkages such as phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Accordingly, oligonucleotides of the disclosure can be stabilized against nucleolytic degradation such as by the incorporation of a modification, e.g., a nucleotide modification.

[000349] In some embodiments, an oligonucleotide may be of up to 50 or up to 100 nucleotides in length in which 2 to 10, 2 to 15, 2 to 16, 2 to 17, 2 to 18, 2 to 19, 2 to 20, 2 to 25, 2 to 30, 2 to 40, 2 to 45, or more nucleotides of the oligonucleotide are modified nucleotides. The oligonucleotide may be of 8 to 30 nucleotides in length in which 2 to 10, 2 to 15, 2 to 16, 2 to 17, 2 to 18, 2 to 19, 2 to 20, 2 to 25, 2 to 30 nucleotides of the oligonucleotide are modified nucleotides. The oligonucleotide may be of 8 to 15 nucleotides in length in which 2 to 4, 2 to 5, 2 to 6, 2 to 7, 2 to 8, 2 to 9, 2 to 10, 2 to 11, 2 to 12, 2 to 13, 2 to 14

nucleotides of the oligonucleotide are modified nucleotides. Optionally, the oligonucleotides may have every nucleotide except 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotides modified. Oligonucleotide modifications are described further herein.

c. Modified Nucleosides

[000350] In some embodiments, the oligonucleotide described herein comprises at least one nucleoside modified at the 2' position of the sugar. In some embodiments, an oligonucleotide comprises at least one 2'-modified nucleoside. In some embodiments, all of the nucleosides in the oligonucleotide are 2'-modified nucleosides.

[000351] In some embodiments, the oligonucleotide described herein comprises one or more non-bicyclic 2'-modified nucleosides, e.g., 2'-deoxy, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleoside.

[000352] In some embodiments, the oligonucleotide described herein comprises one or more 2'-4' bicyclic nucleosides in which the ribose ring comprises a bridge moiety connecting two atoms in the ring, *e.g.*, connecting the 2'-O atom to the 4'-C atom via a methylene (LNA) bridge, an ethylene (ENA) bridge, or a (S)-constrained ethyl (cEt) bridge. Examples of LNAs are described in International Patent Application Publication WO/2008/043753, published on April 17, 2008, and entitled "*RNA Antagonist Compounds For The Modulation Of PCSK9*", the contents of which are incorporated herein by reference in its entirety. Examples of ENAs are provided in International Patent Publication No. WO 2005/042777, published on May 12, 2005, and entitled "*APP/ENA Antisense*"; Morita et al., Nucleic Acid Res., Suppl 1:241-242, 2001; Surono et al., Hum. Gene Ther., 15:749-757, 2004; Koizumi, Curr. Opin. Mol. Ther., 8:144-149, 2006 and Horie et al., Nucleic Acids Symp. Ser (Oxf), 49:171-172, 2005; the disclosures of which are incorporated herein by reference in their entireties. Examples of cEt are provided in US Patents 7,101,993; 7,399,845 and 7,569,686, each of which is herein incorporated by reference in its entirety.

[000353] In some embodiments, the oligonucleotide comprises a modified nucleoside disclosed in one of the following United States Patent or Patent Application Publications: US Patent 7,399,845, issued on July 15, 2008, and entitled "6-*Modified Bicyclic Nucleic Acid Analogs*"; US Patent 7,741,457, issued on June 22, 2010, and entitled "*6-Modified Bicyclic Nucleic Acid Analogs*"; US Patent 8,022,193, issued on September 20, 2011, and entitled "*6-Modified Bicyclic Nucleic Acid Analogs*"; US Patent 7,569,686, issued on August 4, 2009, and

entitled "*Compounds And Methods For Synthesis Of Bicyclic Nucleic Acid Analogs*"; US Patent 7,335,765, issued on February 26, 2008, and entitled "*Novel Nucleoside And Oligonucleotide Analogues*"; US Patent 7,314,923, issued on January 1, 2008, and entitled "*Novel Nucleoside And Oligonucleotide Analogues*"; US Patent 7,816,333, issued on October 19, 2010, and entitled "*Oligonucleotide Analogues And Methods Utilizing The Same*" and US Publication Number 2011/0009471 now US Patent 8,957,201, issued on February 17, 2015, and entitled "*Oligonucleotide Analogues And Methods Utilizing The Same*", the entire contents of each of which are incorporated herein by reference for all purposes.

[000354] In some embodiments, the oligonucleotide comprises at least one modified nucleoside that results in an increase in Tm of the oligonucleotide in a range of 1°C, 2 °C, 3°C, 4 °C, or 5°C compared with an oligonucleotide that does not have the at least one modified nucleoside . The oligonucleotide may have a plurality of modified nucleosides that result in a total increase in Tm of the oligonucleotide in a range of 2 °C, 3 °C, 4 °C, 5 °C, 6 °C, 7 °C, 8 °C, 9 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, 40 °C, 45 °C or more compared with an oligonucleotide that does not have the modified nucleoside.

[000355] The oligonucleotide may comprise a mix of nucleosides of different kinds. For example, an oligonucleotide may comprise a mix of 2'-deoxyribonucleosides or ribonucleosides and 2'-fluoro modified nucleosides. An oligonucleotide may comprise a mix of deoxyribonucleosides or ribonucleosides and 2'-O-Me modified nucleosides. An oligonucleotide may comprise a mix of 2'-fluoro modified nucleosides and 2'-O-Me modified nucleosides. An oligonucleotide may comprise a mix of 2'-4' bicyclic nucleosides and 2'-MOE, 2'-fluoro, or 2'-O-Me modified nucleosides. An oligonucleotide may comprise a mix of non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE, 2'-fluoro, or 2'-O-Me) and 2'-4' bicyclic nucleosides (e.g., LNA, ENA, cEt).

[000356] The oligonucleotide may comprise alternating nucleosides of different kinds. For example, an oligonucleotide may comprise alternating 2'-deoxyribonucleosides or ribonucleosides and 2'-fluoro modified nucleosides. An oligonucleotide may comprise alternating deoxyribonucleosides or ribonucleosides and 2'-O-Me modified nucleosides. An oligonucleotide may comprise alternating 2'-fluoro modified nucleosides and 2'-O-Me modified nucleosides. An oligonucleotide may comprise alternating 2'-4' bicyclic nucleosides and 2'-MOE, 2'-fluoro, or 2'-O-Me modified nucleosides. An oligonucleotide may comprise alternating non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE, 2'-fluoro, or 2'-O-Me) and 2'-4' bicyclic nucleosides (e.g., LNA, ENA, cEt).

[000357] In some embodiments, an oligonucleotide described herein comprises a 5′-vinylphosphonate modification, one or more abasic residues, and/or one or more inverted abasic residues.

d. Internucleoside Linkages / Backbones

[000358] In some embodiments, oligonucleotide may contain a phosphorothioate or other modified internucleoside linkage. In some embodiments, the oligonucleotide comprises phosphorothioate internucleoside linkages. In some embodiments, the oligonucleotide comprises phosphorothioate internucleoside linkages between at least two nucleotides. In some embodiments, the oligonucleotide comprises phosphorothioate internucleoside linkages between all nucleotides. For example, in some embodiments, oligonucleotides comprise modified internucleoside linkages at the first, second, and/or (e.g., and) third internucleoside linkage at the 5' or 3' end of the nucleotide sequence.

[000359] Phosphorus-containing linkages that may be used include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'; see US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5, 177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455, 233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563, 253; 5,571,799; 5,587,361; and 5,625,050.

[000360] In some embodiments, oligonucleotides may have heteroatom backbones, such as methylene(methylimino) or MMI backbones; amide backbones (see De Mesmaeker et al. Ace. Chem. Res. 1995, 28:366-374); morpholino backbones (see Summerton and Weller, U.S. Pat. No. 5,034,506); or peptide nucleic acid (PNA) backbones (wherein the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone, see Nielsen et al., Science 1991, 254, 1497).

e. Stereospecific Oligonucleotides

[000361] In some embodiments, internucleotidic phosphorus atoms of oligonucleotides are chiral, and the properties of the oligonucleotides by adjusted based on the configuration of the chiral phosphorus atoms. In some embodiments, appropriate methods may be used to synthesize P-chiral oligonucleotide analogs in a stereocontrolled manner (e.g., as described in Oka N, Wada T, Stereocontrolled synthesis of oligonucleotide analogs containing chiral internucleotidic phosphorus atoms. Chem Soc Rev. 2011 Dec;40(12):5829-43.) In some embodiments, phosphorothioate containing oligonucleotides comprise nucleoside units that are joined together by either substantially all Sp or substantially all Rp phosphorothioate intersugar linkages are provided. In some embodiments, such phosphorothioate oligonucleotides having substantially chirally pure intersugar linkages are prepared by enzymatic or chemical synthesis, as described, for example, in US Patent 5,587,261, issued on December 12, 1996, the contents of which are incorporated herein by reference in their entirety. In some embodiments, chirally controlled oligonucleotides provide selective cleavage patterns of a target nucleic acid. For example, in some embodiments, a chirally controlled oligonucleotide provides single site cleavage within a complementary sequence of a nucleic acid, as described, for example, in US Patent Application Publication 20170037399 A1, published on February 2, 2017, entitled "CHIRAL DESIGN", the contents of which are incorporated herein by reference in their entirety.

f. Morpholinos

[000362] In some embodiments, the oligonucleotide may be a morpholino-based compounds. Morpholino-based oligomeric compounds are described in Dwaine A. Braasch and David R. Corey, Biochemistry, 2002, 41(14), 4503-4510); Genesis, volume 30, issue 3, 2001; Heasman, J., Dev. Biol., 2002, 243, 209-214; Nasevicius et al., Nat. Genet., 2000, 26, 216-220; Lacerra et al., Proc. Natl. Acad. Sci., 2000, 97, 9591-9596; and U.S. Pat. No. 5,034,506, issued Jul. 23, 1991. In some embodiments, the morpholino-based oligomeric compound is a phosphorodiamidate morpholino oligomer (PMO) (e.g., as described in Iverson, Curr. Opin. Mol. Ther., 3:235-238, 2001; and Wang et al., J. Gene Med., 12:354-364, 2010; the disclosures of which are incorporated herein by reference in their entireties).

g. Peptide Nucleic Acids (PNAs)

[000363] In some embodiments, both a sugar and an internucleoside linkage (the backbone) of the nucleotide units of an oligonucleotide are replaced with novel groups. In some embodiments, the base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an oligonucleotide mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid

(PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide containing backbone, for example, an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative publication that report the preparation of PNA compounds include, but are not limited to, US patent nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen *et al.*, Science, 1991, 254, 1497-1500.

h. Gapmers

[000364] In some embodiments, an oligonucleotide described herein is a gapmer. A gapmer oligonucleotide generally has the formula 5'-X-Y-Z-3', with X and Z as flanking regions around a gap region Y. In some embodiments, flanking region X of formula 5'-X-Y-Z-3' is also referred to as X region, flanking sequence X, 5' wing region X, or 5' wing segment. In some embodiments, flanking region Z of formula 5'-X-Y-Z-3' is also referred to as Z region, flanking sequence Z, 3' wing region Z, or 3' wing segment. In some embodiments, gap region Y of formula 5'-X-Y-Z-3' is also referred to as Y region, Y segment, or gap-segment Y. In some embodiments, each nucleoside in the gap region Y is a 2'-deoxyribonucleoside, and neither the 5' wing region X or the 3' wing region Z contains any 2'-deoxyribonucleosides.

[000365] In some embodiments, the Y region is a contiguous stretch of nucleotides, e.g., a region of 6 or more DNA nucleotides, which are capable of recruiting an RNAse, such as RNAse H. In some embodiments, the gapmer binds to the target nucleic acid, at which point an RNAse is recruited and can then cleave the target nucleic acid. In some embodiments, the Y region is flanked both 5' and 3' by regions X and Z comprising high-affinity modified nucleosides, e.g., one to six high-affinity modified nucleosides. Examples of high affinity modified nucleosides include, but are not limited to, 2'-modified nucleosides (e.g., 2'-MOE, 2'O-Me, 2'-F) or 2'-4' bicyclic nucleosides (e.g., LNA, cEt, ENA). In some embodiments, the flanking sequences X and Z may be of 1-20 nucleotides, 1-8 nucleotides, or 1-5 nucleotides in length. The flanking sequences X and Z may be of similar length or of dissimilar lengths. In some embodiments, the gap-segment Y may be a nucleotide sequence of 5-20 nucleotides, 5-15 twelve nucleotides, or 6-10 nucleotides in length.

[000366] In some embodiments, the gap region of the gapmer oligonucleotides may contain modified nucleotides known to be acceptable for efficient RNase H action in addition to DNA nucleotides, such as C4'-substituted nucleotides, acyclic nucleotides, and arabino-configured nucleotides. In some embodiments, the gap region comprises one or more unmodified internucleosides. In some embodiments, one or both flanking regions each

independently comprise one or more phosphorothioate internucleoside linkages (e.g., phosphorothioate internucleoside linkages or other linkages) between at least two, at least three, at least four, at least five or more nucleotides. In some embodiments, the gap region and two flanking regions each independently comprise modified internucleoside linkages (e.g., phosphorothioate internucleoside linkages or other linkages) between at least two, at least three, at least four, at least five or more nucleotides.

[000367] A gapmer may be produced using appropriate methods. Representative U.S. patents, U.S. patent publications, and PCT publications that teach the preparation of gapmers include, but are not limited to, U.S. Pat. Nos. 5,013,830; 5,149,797; 5,220,007; 5,256,775; 5,366,878; 5,403,711; 5,491,133; 5,565,350; 5,623,065; 5,652,355; 5,652,356; 5,700,922; 5,898,031; 7,015,315; 7,101,993; 7,399,845; 7,432,250; 7,569,686; 7,683,036; 7,750,131; 8,580,756; 9,045,754; 9,428,534; 9,695,418; 10,017,764; 10,260,069; 9,428,534; 8,580,756; U.S. patent publication Nos. US20050074801, US20090221685; US20090286969, US20100197762, and US20110112170; PCT publication Nos. WO2004069991; WO2005023825; WO2008049085 and WO2009090182; and EP Patent No. EP2,149,605, each of which is herein incorporated by reference in its entirety.

[000368] In some embodiments, a gapmer is 10-40 nucleosides in length. For example, a gapmer may be 10-40, 10-35, 10-30, 10-25, 10-20, 10-15, 15-40, 15-35, 15-30, 15-25, 15-20, 20-40, 20-35, 20-30, 20-25, 25-40, 25-35, 25-30, 30-40, 30-35, or 35-40 nucleosides in length. In some embodiments, a gapmer is 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 nucleosides in length.

[000369] In some embodiments, the gap region Y in a gapmer is 5-20 nucleosides in length. For example, the gap region Y may be 5-20, 5-15, 5-10, 10-20, 10-15, or 15-20 nucleosides in length. In some embodiments, the gap region Y is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleosides in length. In some embodiments, each nucleoside in the gap region Y is a 2'-deoxyribonucleoside. In some embodiments, all nucleosides in the gap region Y are 2'-deoxyribonucleosides. In some embodiments, one or more of the nucleosides in the gap region Y is a modified nucleoside (e.g., a 2' modified nucleoside such as those described herein). In some embodiments, one or more cytosines in the gap region Y are optionally 5-methyl-cytosines. In some embodiments, each cytosine in the gap region Y is a 5-methyl-cytosines.

[000370] In some embodiments, the 5'wing region of a gapmer (X in the 5'-X-Y-Z-3' formula) and the 3'wing region of a gapmer (Z in the 5'-X-Y-Z-3' formula) are independently 1-20 nucleosides long. For example, the 5'wing region of a gapmer (X in the 5'-X-Y-Z-3'

formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula) may be independently 1-20, 1-15, 1-10, 1-7, 1-5, 1-3, 1-2, 2-5, 2-7, 3-5, 3-7, 5-20, 5-15, 5-10, 10-20, 10-15, or 15-20 nucleosides long. In some embodiments, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3′ formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula) are independently 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleosides long. In some embodiments, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3′ formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula) are of the same length. In some embodiments, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3′ formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula) are of different lengths. In some embodiments, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3′ formula) is longer than the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula). In some embodiments, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3′ formula) is shorter than the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula).

[000371] In some embodiments, a gapmer comprises a 5'-X-Y-Z-3′ of 5-10-5, 4-12-4, 3-14-3, 2-16-2, 1-18-1, 3-10-3, 2-10-2, 1-10-1, 2-8-2, 4-6-4, 3-6-3, 2-6-2, 4-7-4, 3-7-3, 2-7-2, 4-8-4, 3-8-3, 2-8-2, 1-8-1, 2-9-2, 1-9-1, 2-10-2, 1-10-1, 1-12-1, 1-16-1, 2-15-1, 1-15-2, 1-14-3, 3-14-1, 2-14-2, 1-13-4, 4-13-1, 2-13-3, 3-13-2, 1-12-5, 5-12-1, 2-12-4, 4-12-2, 3-12-3, 1-11-6, 6-11-1, 2-11-5, 5-11-2, 3-11-4, 4-11-3, 1-17-1, 2-16-1, 1-16-2, 1-15-3, 3-15-1, 2-15-2, 1-14-4, 4-14-1, 2-14-3, 3-14-2, 1-13-5, 5-13-1, 2-13-4, 4-13-2, 3-13-3, 1-12-6, 6-12-1, 2-12-5, 5-12-2, 3-12-4, 4-12-3, 1-11-7, 7-11-1, 2-11-6, 6-11-2, 3-11-5, 5-11-3, 4-11-4, 1-18-1, 1-17-2, 2-17-1, 1-16-3, 1-16-3, 2-16-2, 1-15-4, 4-15-1, 2-15-3, 3-15-2, 1-14-5, 5-14-1, 2-14-4, 4-14-2, 3-14-3, 1-13-6, 6-13-1, 2-13-5, 5-13-2, 3-13-4, 4-13-3, 1-12-7, 7-12-1, 2-12-6, 6-12-2, 3-12-5, 5-12-3, 1-11-8, 8-11-1, 2-11-7, 7-11-2, 3-11-6, 6-11-3, 4-11-5, 5-11-4, 1-18-1, 1-17-2, 2-17-1, 1-16-3, 3-16-1, 2-16-2, 1-15-4, 4-15-1, 2-15-3, 3-15-2, 1-14-5, 2-14-4, 4-14-2, 3-14-3, 1-13-6, 6-13-1, 2-13-5, 5-13-2, 3-13-4, 4-13-3, 1-12-7, 7-12-1, 2-12-6, 6-12-2, 3-12-5, 5-12-3, 1-11-8, 8-11-1, 2-11-7, 7-11-2, 3-11-6, 6-11-3, 4-11-5, 5-11-4, 1-19-1, 1-18-2, 2-18-1, 1-17-3, 3-17-1, 2-17-2, 1-16-4, 4-16-1, 2-16-3, 3-16-2, 1-15-5, 2-15-4, 4-15-2, 3-15-3, 1-14-6, 6-14-1, 2-14-5, 5-14-2, 3-14-4, 4-14-3, 1-13-7, 7-13-1, 2-13-6, 6-13-2, 3-13-5, 5-13-3, 4-13-4, 1-12-8, 8-12-1, 2-12-7, 7-12-2, 3-12-6, 6-12-3, 4-12-5, 5-12-4, 2-11-8, 8-11-2, 3-11-7, 7-11-3, 4-11-6, 6-11-4, 5-11-5, 1-20-1, 1-19-2, 2-19-1, 1-18-3, 3-18-1, 2-18-2, 1-17-4, 4-17-1, 2-17-3, 3-17-2, 1-16-5, 2-16-4, 4-16-2, 3-16-3, 1-15-6, 6-15-1, 2-15-5, 5-15-2, 3-15-4, 4-15-3, 1-14-7, 7-14-1, 2-14-6, 6-14-2, 3-14-5, 5-14-3, 4-14-4, 1-13-8, 8-13-1, 2-13-7, 7-13-2, 3-13-6, 6-13-3, 4-13-5, 5-13-4, 2-12-8, 8-12-2, 3-12-7, 7-12-3, 4-12-6, 6-12-4, 5-12-5, 3-11-8, 8-11-3, 4-11-7, 7-11-4, 5-11-6, 6-11-5, 1-21-1, 1-20-2, 2-20-1, 1-20-3, 3-19-1, 2-19-2, 1-18-4, 4-18-1, 2-18-3, 3-18-2, 1-17-5, 2-17-4, 4-

17-2, 3-17-3, 1-16-6, 6-16-1, 2-16-5, 5-16-2, 3-16-4, 4-16-3, 1-15-7, 7-15-1, 2-15-6, 6-15-2, 3-15-5, 5-15-3, 4-15-4, 1-14-8, 8-14-1, 2-14-7, 7-14-2, 3-14-6, 6-14-3, 4-14-5, 5-14-4, 2-13-8, 8-13-2, 3-13-7, 7-13-3, 4-13-6, 6-13-4, 5-13-5, 1-12-10, 10-12-1, 2-12-9, 9-12-2, 3-12-8, 8-12-3, 4-12-7, 7-12-4, 5-12-6, 6-12-5, 4-11-8, 8-11-4, 5-11-7, 7-11-5, 6-11-6, 1-22-1, 1-21-2, 2-21-1, 1-21-3, 3-20-1, 2-20-2, 1-19-4, 4-19-1, 2-19-3, 3-19-2, 1-18-5, 2-18-4, 4-18-2, 3-18-3, 1-17-6, 6-17-1, 2-17-5, 5-17-2, 3-17-4, 4-17-3, 1-16-7, 7-16-1, 2-16-6, 6-16-2, 3-16-5, 5-16-3, 4-16-4, 1-15-8, 8-15-1, 2-15-7, 7-15-2, 3-15-6, 6-15-3, 4-15-5, 5-15-4, 2-14-8, 8-14-2, 3-14-7, 7-14-3, 4-14-6, 6-14-4, 5-14-5, 3-13-8, 8-13-3, 4-13-7, 7-13-4, 5-13-6, 6-13-5, 4-12-8, 8-12-4, 5-12-7, 7-12-5, 6-12-6, 5-11-8, 8-11-5, 6-11-7, or 7-11-6. The numbers indicate the number of nucleosides in X, Y, and Z regions in the 5'-X-Y-Z-3′ gapmer.

[000372] In some embodiments, one or more nucleosides in the 5'wing region of a gapmer (X in the 5'-X-Y-Z-3′ formula) or the 3'wing region of a gapmer (Z in the 5'-X-Y-Z-3′ formula) are modified nucleotides (e.g., high-affinity modified nucleosides). In some embodiments, the modified nuclsoside (e.g., high-affinity modified nucleosides) is a 2'-modifeid nucleoside. In some embodiments, the 2'-modified nucleoside is a 2'-4' bicyclic nucleoside or a non-bicyclic 2'-modified nucleoside. In some embodiments, the high-affinity modified nucleoside is a 2'-4' bicyclic nucleoside (e.g., LNA, cEt, or ENA) or a non-bicyclic 2'-modified nucleoside (e.g., 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA)).

[000373] In some embodiments, one or more nucleosides in the 5'wing region of a gapmer (X in the 5'-X-Y-Z-3′ formula) are high-affinity modified nucleosides. In some embodiments, each nucleoside in the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3′ formula) is a high-affinity modified nucleoside. In some embodiments, one or more nucleosides in the 3'wing region of a gapmer (Z in the 5'-X-Y-Z-3′ formula) are high-affinity modified nucleosides. In some embodiments, each nucleoside in the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula) is a high-affinity modified nucleoside. In some embodiments, one or more nucleosides in the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3′ formula) are high-affinity modified nucleosides and one or more nucleosides in the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula) are high-affinity modified nucleosides. In some embodiments, each nucleoside in the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3′ formula) is a high-affinity modified nucleoside and each nucleoside in the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3′ formula) is high-affinity modified nucleoside.

[000374] In some embodiments, the 5'wing region of a gapmer (X in the 5'-X-Y-Z-3' formula) comprises the same high affinity nucleosides as the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula). For example, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) may comprise one or more non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me). In another example, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) may comprise one or more 2'-4' bicyclic nucleosides (e.g., LNA or cEt). In some embodiments, each nucleoside in the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) is a non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me). In some embodiments, each nucleoside in the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) is a 2'-4' bicyclic nucleosides (e.g., LNA or cEt).

[000375] In some embodiments, a gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 1-7 (e.g., 1, 2, 3, 4, 5, 6, or 7) nucleosides in length and Y is 6-10 (e.g., 6, 7, 8, 9, or 10) nucleosides in length, wherein each nucleoside in X and Z is a non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me) and each nucleoside in Y is a 2'-deoxyribonucleoside. In some embodiments, the gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 1-7 (e.g., 1, 2, 3, 4, 5, 6, or 7) nucleosides in length and Y is 6-10 (e.g., 6, 7, 8, 9, or 10) nucleosides in length, wherein each nucleoside in X and Z is a 2'-4' bicyclic nucleosides (e.g., LNA or cEt) and each nucleoside in Y is a 2'-deoxyribonucleoside. In some embodiments, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) comprises different high affinity nucleosides as the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula). For example, the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) may comprise one or more non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) may comprise one or more 2'-4' bicyclic nucleosides (e.g., LNA or cEt). In another example, the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) may comprise one or more non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me) and the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) may comprise one or more 2'-4' bicyclic nucleosides (e.g., LNA or cEt).

[000376] In some embodiments, a gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 1-7 (e.g., 1, 2, 3, 4, 5, 6, or 7) nucleosides in length and Y is 6-10 (e.g., 6, 7, 8, 9, or 10) nucleosides in length, wherein each nucleoside in X is a non-

bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me), each nucleoside in Z is a 2'-4' bicyclic nucleosides (e.g., LNA or cEt), and each nucleoside in Y is a 2'-deoxyribonucleoside. In some embodiments, the gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 1-7 (e.g., 1, 2, 3, 4, 5, 6, or 7) nucleosides in length and Y is 6-10 (e.g., 6, 7, 8, 9, or 10) nucleosides in length, wherein each nucleoside in X is a 2'-4' bicyclic nucleosides (e.g., LNA or cEt), each nucleoside in Z is a non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me) and each nucleoside in Y is a 2'-deoxyribonucleoside.

[000377] In some embodiments, the 5'wing region of a gapmer (X in the 5'-X-Y-Z-3' formula) comprises one or more non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me) and one or more 2'-4' bicyclic nucleosides (e.g., LNA or cEt). In some embodiments, the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) comprises one or more non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me) and one or more 2'-4' bicyclic nucleosides (e.g., LNA or cEt). In some embodiments, both the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) comprise one or more non-bicyclic 2'-modified nucleosides (e.g., 2'-MOE or 2'-O-Me) and one or more 2'-4' bicyclic nucleosides (e.g., LNA or cEt).

[000378] In some embodiments, a gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 2-7 (e.g., 2, 3, 4, 5, 6, or 7) nucleosides in length and Y is 6-10 (e.g., 6, 7, 8, 9, or 10) nucleosides in length, wherein at least one but not all (e.g., 1, 2, 3, 4, 5, or 6) of positions 1, 2, 3, 4, 5, 6, or 7 in X (the 5' most position is position 1) is a non-bicyclic 2'-modified nucleoside (e.g., 2'-MOE or 2'-O-Me), wherein the rest of the nucleosides in both X and Z are 2'-4' bicyclic nucleosides (e.g., LNA or cEt), and wherein each nucleoside in Y is a 2'deoxyribonucleoside. In some embodiments, the gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 2-7 (e.g., 2, 3, 4, 5, 6, or 7) nucleosides in length and Y is 6-10 (e.g., 6, 7, 8, 9, or 10) nucleosides in length, wherein at least one but not all (e.g., 1, 2, 3, 4, 5, or 6) of positions 1, 2, 3, 4, 5, 6, or 7 in Z (the 5' most position is position 1) is a non-bicyclic 2'-modified nucleoside (e.g., 2'-MOE or 2'-O-Me), wherein the rest of the nucleosides in both X and Z are 2'-4' bicyclic nucleosides (e.g., LNA or cEt), and wherein each nucleoside in Y is a 2'deoxyribonucleoside. In some embodiments, the gapmer comprises a 5'-X-Y-Z-3' configuration, wherein X and Z is independently 2-7 (e.g., 2, 3, 4, 5, 6, or 7) nucleosides in length and Y is 6-10 (e.g., 6, 7, 8, 9, or 10) nucleosides in length, wherein at least one but not all (e.g., 1, 2, 3, 4, 5, or 6) of positions 1, 2, 3, 4, 5, 6, or 7 in X and at least one of positions but not all (e.g., 1, 2, 3, 4, 5, or 6) 1, 2, 3, 4, 5, 6, or 7 in Z (the 5' most position is position 1) is a non-bicyclic 2'-modified nucleoside (e.g., 2'-MOE or 2'-O-Me),

wherein the rest of the nucleosides in both X and Z are 2'-4' bicyclic nucleosides (e.g., LNA or cEt), and wherein each nucleoside in Y is a 2'deoxyribonucleoside.

[000379] Non-limiting examples of gapmers configurations with a mix of non-bicyclic 2'-modified nucleoside (e.g., 2'-MOE or 2'-O-Me) and 2'-4' bicyclic nucleosides (e.g., LNA or cEt) in the 5'wing region of the gapmer (X in the 5'-X-Y-Z-3' formula) and/or the 3'wing region of the gapmer (Z in the 5'-X-Y-Z-3' formula) include: BBB-(D)n-BBBAA; KKK-(D)n-KKKAA; LLL-(D)n-LLLAA; BBB-(D)n-BBBEE; KKK-(D)n-KKKEE; LLL-(D)n-LLLEE; BBB-(D)n-BBBAA; KKK-(D)n-KKKAA; LLL-(D)n-LLLAA; BBB-(D)n-BBBEE; KKK-(D)n-KKKEE; LLL-(D)n-LLLEE; BBB-(D)n-BBBAAA; KKK-(D)n-KKKAAA; LLL-(D)n-LLLAAA; BBB-(D)n-BBBEEE; KKK-(D)n-KKKEEE; LLL-(D)n-LLLEEE; BBB-(D)n-BBBAAA; KKK-(D)n-KKKAAA; LLL-(D)n-LLLAAA; BBB-(D)n-BBBEEE; KKK-(D)n-KKKEEE; LLL-(D)n-LLLEEE; BABA-(D)n-ABAB; KAKA-(D)n-AKAK; LALA-(D)n-ALAL; BEBE-(D)n-EBEB; KEKE-(D)n-EKEK; LELE-(D)n-ELEL; BABA-(D)n-ABAB; KAKA-(D)n-AKAK; LALA-(D)n-ALAL; BEBE-(D)n-EBEB; KEKE-(D)n-EKEK; LELE-(D)n-ELEL; ABAB-(D)n-ABAB; AKAK-(D)n-AKAK; ALAL-(D)n-ALAL; EBEB-(D)n-EBEB; EKEK-(D)n-EKEK; ELEL-(D)n-ELEL; ABAB-(D)n-ABAB; AKAK-(D)n-AKAK; ALAL-(D)n-ALAL; EBEB-(D)n-EBEB; EKEK-(D)n-EKEK; ELEL-(D)n-ELEL; AABB-(D)n-BBAA; BBAA-(D)n-AABB; AAKK-(D)n-KKAA; AALL-(D)n-LLAA; EEBB-(D)n-BBEE; EEKK-(D)n-KKEE; EELL-(D)n-LLEE; AABB-(D)n-BBAA; AAKK-(D)n-KKAA; AALL-(D)n-LLAA; EEBB-(D)n-BBEE; EEKK-(D)n-KKEE; EELL-(D)n-LLEE; BBB-(D)n-BBA; KKK-(D)n-KKA; LLL-(D)n-LLA; BBB-(D)n-BBE; KKK-(D)n-KKE; LLL-(D)n-LLE; BBB-(D)n-BBA; KKK-(D)n-KKA; LLL-(D)n-LLA; BBB-(D)n-BBE; KKK-(D)n-KKE; LLL-(D)n-LLE; BBB-(D)n-BBA; KKK-(D)n-KKA; LLL-(D)n-LLA; BBB-(D)n-BBE; KKK-(D)n-KKE; LLL-(D)n-LLE; ABBB-(D)n-BBBA; AKKK-(D)n-KKKA; ALLL-(D)n-LLLA; EBBB-(D)n-BBBE; EKKK-(D)n-KKKE; ELLL-(D)n-LLLE; ABBB-(D)n-BBBA; AKKK-(D)n-KKKA; ALLL-(D)n-LLLA; EBBB-(D)n-BBBE; EKKK-(D)n-KKKE; ELLL-(D)n-LLLE; ABBB-(D)n-BBBAA; AKKK-(D)n-KKKAA; ALLL-(D)n-LLLAA; EBBB-(D)n-BBBEE; EKKK-(D)n-KKKEE; ELLL-(D)n-LLLEE; ABBB-(D)n-BBBAA; AKKK-(D)n-KKKAA; ALLL-(D)n-LLLAA; EBBB-(D)n-BBBEE; EKKK-(D)n-KKKEE; ELLL-(D)n-LLLEE; AABBB-(D)n-BBB; AAKKK-(D)n-KKK; AALLL-(D)n-LLL; EEBBB-(D)n-BBB; EEKKK-(D)n-KKK; EELLL-(D)n-LLL; AABBB-(D)n-BBB; AAKKK-(D)n-KKK; AALLL-(D)n-LLL; EEBBB-(D)n-BBB; EEKKK-(D)n-KKK; EELLL-(D)n-LLL; AABBB-(D)n-BBBA; AAKKK-(D)n-KKKA; AALLL-(D)n-LLLA; EEBBB-(D)n-BBBE; EEKKK-(D)n-KKKE; EELLL-(D)n-LLLE; AABBB-(D)n-BBBA; AAKKK-(D)n-KKKA; AALLL-(D)n-LLLA; EEBBB-

(D)n-BBBE; EEKKK-(D)n-KKKE; EELLL-(D)n-LLLE; ABBAABB-(D)n-BB; AKKAAKK-(D)n-KK; ALLAALLL-(D)n-LL; EBBEEBB-(D)n-BB; EKKEEKK-(D)n-KK; ELLEELL-(D)n-LL; ABBAABB-(D)n-BB; AKKAAKK-(D)n-KK; ALLAALL-(D)n-LL; EBBEEBB-(D)n-BB; EKKEEKK-(D)n-KK; ELLEELL-(D)n-LL; ABBABB-(D)n-BBB; AKKAKK-(D)n-KKK; ALLALLL-(D)n-LLL; EBBEBB-(D)n-BBB; EKKEKK-(D)n-KKK; ELLELL-(D)n-LLL; ABBABB-(D)n-BBB; AKKAKK-(D)n-KKK; ALLALL-(D)n-LLL; EBBEBB-(D)n-BBB; EKKEKK-(D)n-KKK; ELLELL-(D)n-LLL; EEEK-(D)n-EEEEEEEE; EEK-(D)n-EEEEEEEEE; EK-(D)n-EEEEEEEEEE; EK-(D)n-EEEKK; K-(D)n-EEEKEKE; K-(D)n-EEEKEKEE; K-(D)n-EEKEK; EK-(D)n-EEEEKEKE; EK-(D)n-EEEKEK; EEK-(D)n-KEEKE; EK-(D)n-EEKEK; EK-(D)n-KEEK; EEK-(D)n-EEEKEK; EK-(D)n-KEEEKEE; EK-(D)n-EEKEKE; EK-(D)n-EEEKEKE; and EK-(D)n-EEEEKEK;. "A" nucleosides comprise a 2′-modified nucleoside; "B" represents a 2'-4' bicyclic nucleoside; "K" represents a constrained ethyl nucleoside (cEt); "L" represents an LNA nucleoside; and "E" represents a 2′-MOE modified ribonucleoside; "D" represents a 2'-deoxyribonucleoside; "n" represents the length of the gap segment (Y in the 5'-X-Y-Z-3′ configuration) and is an integer between 1-20.

[000380] In some embodiments, any one of the gapmers described herein comprises one or more modified nucleoside linkages (e.g., a phosphorothioate linkage) in each of the X, Y, and Z regions. In some embodiments, each internucleoside linkage in the any one of the gapmers described herein is a phosphorothioate linkage. In some embodiments, each of the X, Y, and Z regions independently comprises a mix of phosphorothioate linkages and phosphodiester linkages. In some embodiments, each internucleoside linkage in the gap region Y is a phosphorothioate linkage, the 5'wing region X comprises a mix of phosphorothioate linkages and phosphodiester linkages, and the 3'wing region Z comprises a mix of phosphorothioate linkages and phosphodiester linkages.

i. Mixmers

[000381] In some embodiments, an oligonucleotide described herein may be a mixmer or comprise a mixmer sequence pattern. In general, mixmers are oligonucleotides that comprise both naturally and non-naturally occurring nucleosides or comprise two different types of non-naturally occurring nucleosides typically in an alternating pattern. Mixmers generally have higher binding affinity than unmodified oligonucleotides and may be used to specifically bind a target molecule, *e.g.*, to block a binding site on the target molecule. Generally, mixmers do not recruit an RNase to the target molecule and thus do not promote cleavage of the target

molecule. Such oligonucleotides that are incapable of recruiting RNase H have been described, for example, see WO2007/112754 or WO2007/112753.

[000382] In some embodiments, the mixmer comprises or consists of a repeating pattern of nucleoside analogues and naturally occurring nucleosides, or one type of nucleoside analogue and a second type of nucleoside analogue. However, a mixmer need not comprise a repeating pattern and may instead comprise any arrangement of modified nucleoside s and naturally occurring nucleoside s or any arrangement of one type of modified nucleoside and a second type of modified nucleoside. The repeating pattern, may, for instance be every second or every third nucleoside is a modified nucleoside, such as LNA, and the remaining nucleoside s are naturally occurring nucleosides, such as DNA, or are a 2′ substituted nucleoside analogue such as 2′-MOE or 2′ fluoro analogues, or any other modified nucleoside described herein. It is recognized that the repeating pattern of modified nucleoside, such as LNA units, may be combined with modified nucleoside at fixed positions—*e.g.* at the 5′ or 3′ termini.

[000383] In some embodiments, a mixmer does not comprise a region of more than 5, more than 4, more than 3, or more than 2 consecutive naturally occurring nucleosides, such as DNA nucleosides. In some embodiments, the mixmer comprises at least a region consisting of at least two consecutive modified nucleoside, such as at least two consecutive LNAs. In some embodiments, the mixmer comprises at least a region consisting of at least three consecutive modified nucleoside units, such as at least three consecutive LNAs.

[000384] In some embodiments, the mixmer does not comprise a region of more than 7, more than 6, more than 5, more than 4, more than 3, or more than 2 consecutive nucleoside analogues, such as LNAs. In some embodiments, LNA units may be replaced with other nucleoside analogues, such as those referred to herein.

[000385] Mixmers may be designed to comprise a mixture of affinity enhancing modified nucleosides, such as in non-limiting example LNA nucleosides and 2'-O-Me nucleosides. In some embodiments, a mixmer comprises modified internucleoside linkages (*e.g.*, phosphorothioate internucleoside linkages or other linkages) between at least two, at least three, at least four, at least five or more nucleosides.

[000386] A mixmer may be produced using any suitable method. Representative U.S. patents, U.S. patent publications, and PCT publications that teach the preparation of mixmers include U.S. patent publication Nos. US20060128646, US20090209748, US20090298916, US20110077288, and US20120322851, and U.S. patent No. 7687617.

[000387] In some embodiments, a mixmer comprises one or more morpholino nucleosides. For example, in some embodiments, a mixmer may comprise morpholino

nucleosides mixed (*e.g.*, in an alternating manner) with one or more other nucleosides (*e.g.*, DNA, RNA nucleosides) or modified nucleosides (*e.g.*, LNA, 2'-O-Me nucleosides).

[000388] In some embodiments, mixmers are useful for splice correcting or exon skipping, for example, as reported in Touznik A., et al., *LNA/DNA mixmer-based antisense oligonucleotides correct alternative splicing of the SMN2 gene and restore SMN protein expression in type 1 SMA fibroblasts* Scientific Reports, volume 7, Article number: 3672 (2017), Chen S. et al., *Synthesis of a Morpholino Nucleic Acid (MNA)-Uridine Phosphoramidite, and Exon Skipping Using MNA/2'-O-Methyl Mixmer Antisense Oligonucleotide*, Molecules 2016, 21, 1582, the contents of each which are incorporated herein by reference.

j. RNA Interference (RNAi)

[000389] In some embodiments, oligonucleotides provided herein may be in the form of small interfering RNAs (siRNA), also known as short interfering RNA or silencing RNA. SiRNA, is a class of double-stranded RNA molecules, typically about 20-25 base pairs in length that target nucleic acids (e.g., mRNAs) for degradation via the RNA interference (RNAi) pathway in cells. Specificity of siRNA molecules may be determined by the binding of the antisense strand of the molecule to its target RNA. Effective siRNA molecules are generally less than 30 to 35 base pairs in length to prevent the triggering of non-specific RNA interference pathways in the cell via the interferon response, although longer siRNA can also be effective. In some embodiments, the siRNA molecules are 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or more base pairs in length. In some embodiments, the siRNA molecules are 8 to 30 base pairs in length, 10 to 15 base pairs in length, 10 to 20 base pairs in length, 15 to 25 base pairs in length, 19 to 21 base pairs in length, 21 to 23 base pairs in length.

[000390] Following selection of an appropriate target RNA sequence, siRNA molecules that comprise a nucleotide sequence complementary to all or a portion of the target sequence, i.e. an antisense sequence, can be designed and prepared using appropriate methods (see, e.g., PCT Publication Number WO 2004/016735; and U.S. Patent Publication Nos. 2004/0077574 and 2008/0081791). The siRNA molecule can be double stranded (i.e. a dsRNA molecule comprising an antisense strand and a complementary sense strand strand that hybridizes to form the dsRNA) or single-stranded (i.e. a ssRNA molecule comprising just an antisense strand). The siRNA molecules can comprise a duplex, asymmetric duplex, hairpin or

asymmetric hairpin secondary structure, having self-complementary sense and antisense strands.

[000391] In some embodiments, the antisense strand of the siRNA molecule is 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or more nucleotides in length. In some embodiments, the antisense strand is 8 to 50 nucleotides in length, 8 to 40 nucleotides in length, 8 to 30 nucleotides in length, 10 to 15 nucleotides in length, 10 to 20 nucleotides in length, 15 to 25 nucleotides in length, 19 to 21 nucleotides in length, 21 to 23 nucleotides in lengths.

[000392] In some embodiments, the sense strand of the siRNA molecule is 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, or more nucleotides in length. In some embodiments, the sense strand is 8 to 50 nucleotides in length, 8 to 40 nucleotides in length, 8 to 30 nucleotides in length, 10 to 15 nucleotides in length, 10 to 20 nucleotides in length, 15 to 25 nucleotides in length, 19 to 21 nucleotides in length, 21 to 23 nucleotides in lengths.

[000393] In some embodiments, siRNA molecules comprise an antisense strand comprising a region of complementarity to a target region in a target mRNA. In some embodiments, the region of complementarity is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99% or 100% complementary to a target region in a target mRNA. In some embodiments, the target region is a region of consecutive nucleotides in the target mRNA. In some embodiments, a complementary nucleotide sequence need not be 100% complementary to that of its target to be specifically hybridizable or specific for a target RNA sequence.

[000394] In some embodiments, siRNA molecules comprise an antisense strand that comprises a region of complementarity to a target RNA sequence and the region of complementarity is in the range of 8 to 15, 8 to 30, 8 to 40, or 10 to 50, or 5 to 50, or 5 to 40 nucleotides in length. In some embodiments, a region of complementarity is 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length. In some embodiments, the region of complementarity is complementary with at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25 or more consecutive nucleotides of a target RNA sequence. In some embodiments, siRNA molecules comprise a nucleotide sequence that contains no more than 1, 2, 3, 4, or 5 base mismatches compared to the portion of the consecutive nucleotides of target RNA

sequence. In some embodiments, siRNA molecules comprise a nucleotide sequence that has up to 3 mismatches over 15 bases, or up to 2 mismatches over 10 bases.

[000395] In some embodiments, siRNA molecules comprise an antisense strand comprising a nucleotide sequence that is complementary (e.g., at least 85%, at least 90%, at least 95%, or 100%) to the target RNA sequence of the oligonucleotides provided herein. In some embodiments, siRNA molecules comprise an antisense strand comprising a nucleotide sequence that is at least 85%, at least 90%, at least 95%, or 100% identical to the oligonucleotides provided herein. In some embodiments, siRNA molecules comprise an antisense strand comprising at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25 or more consecutive nucleotides of the oligonucleotides provided herein.

[000396] Double-stranded siRNA may comprise sense and anti-sense RNA strands that are the same length or different lengths. Double-stranded siRNA molecules can also be assembled from a single oligonucleotide in a stem-loop structure, wherein self-complementary sense and antisense regions of the siRNA molecule are linked by means of a nucleic acid based or non-nucleic acid-based linker(s), as well as circular single-stranded RNA having two or more loop structures and a stem comprising self-complementary sense and antisense strands, wherein the circular RNA can be processed either in vivo or in vitro to generate an active siRNA molecule capable of mediating RNAi. Small hairpin RNA (shRNA) molecules thus are also contemplated herein. These molecules comprise a specific antisense sequence in addition to the reverse complement (sense) sequence, typically separated by a spacer or loop sequence. Cleavage of the spacer or loop provides a single-stranded RNA molecule and its reverse complement, such that they may anneal to form a dsRNA molecule (optionally with additional processing steps that may result in addition or removal of one, two, three or more nucleotides from the 3' end and/or (e.g., and) the 5' end of either or both strands). A spacer can be of a sufficient length to permit the antisense and sense sequences to anneal and form a double-stranded structure (or stem) prior to cleavage of the spacer (and, optionally, subsequent processing steps that may result in addition or removal of one, two, three, four, or more nucleotides from the 3' end and/or (e.g., and) the 5' end of either or both strands). A spacer sequence is may be an unrelated nucleotide sequence that is situated between two complementary nucleotide sequence regions which, when annealed into a double-stranded nucleic acid, comprise a shRNA.

[000397] The overall length of the siRNA molecules can vary from about 14 to about 100 nucleotides depending on the type of siRNA molecule being designed. Generally between about 14 and about 50 of these nucleotides are complementary to the RNA target sequence, i.e. constitute the specific antisense sequence of the siRNA molecule. For example, when the siRNA is a double- or single-stranded siRNA, the length can vary from about 14 to about 50 nucleotides, whereas when the siRNA is a shRNA or circular molecule, the length can vary from about 40 nucleotides to about 100 nucleotides.

[000398] An siRNA molecule may comprise a 3' overhang at one end of the molecule, The other end may be blunt-ended or have also an overhang (5' or 3'). When the siRNA molecule comprises an overhang at both ends of the molecule, the length of the overhangs may be the same or different. In one embodiment, the siRNA molecule of the present disclosure comprises 3' overhangs of about 1 to about 3 nucleotides on both ends of the molecule. In some embodiments, the siRNA molecule comprises 3' overhangs of about 1 to about 3 nucleotides on the sense strand. In some embodiments, the siRNA molecule comprises 3' overhangs of about 1 to about 3 nucleotides on the antisense strand. In some embodiments, the siRNA molecule comprises 3' overhangs of about 1 to about 3 nucleotides on both the sense strand and the antisense strand.

[000399] In some embodiments, the siRNA molecule comprises one or more modified nucleotides (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more). In some embodiments, the siRNA molecule comprises one or more modified nucleotides and/or (e.g., and) one or more modified internucleotide linkages. In some embodiments, the modified nucleotide is a modified sugar moiety (e.g. a 2' modified nucleotide). In some embodiments, the siRNA molecule comprises one or more 2' modified nucleotides, e.g., a 2'-deoxy, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O--N-methylacetamido (2'-O--NMA). In some embodiments, each nucleotide of the siRNA molecule is a modified nucleotide (e.g., a 2'-modified nucleotide). In some embodiments, the siRNA molecule comprises one or more phosphorodiamidate morpholinos. In some embodiments, each nucleotide of the siRNA molecule is a phosphorodiamidate morpholino.

[000400] In some embodiments, the siRNA molecule contains a phosphorothioate or other modified internucleotide linkage. In some embodiments, the siRNA molecule comprises phosphorothioate internucleoside linkages. In some embodiments, the siRNA molecule comprises phosphorothioate internucleoside linkages between at least two nucleotides. In

some embodiments, the siRNA molecule comprises phosphorothioate internucleoside linkages between all nucleotides. For example, in some embodiments, the siRNA molecule comprises modified internucleotide linkages at the first, second, and/or (e.g., and) third internucleoside linkage at the 5' or 3' end of the siRNA molecule.

[000401] In some embodiments, the modified internucleotide linkages are phosphorus-containing linkages. In some embodiments, phosphorus-containing linkages that may be used include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'; see US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5, 177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455, 233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563, 253; 5,571,799; 5,587,361; and 5,625,050.

[000402] Any of the modified chemistries or formats of siRNA molecules described herein can be combined with each other. For example, one, two, three, four, five, or more different types of modifications can be included within the same siRNA molecule.

[000403] In some embodiments, the antisense strand comprises one or more modified nucleotides (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more). In some embodiments, the antisense strand comprises one or more modified nucleotides and/or (e.g., and) one or more modified internucleotide linkages. In some embodiments, the modified nucleotide comprises a modified sugar moiety (e.g. a 2' modified nucleotide). In some embodiments, the antisense strand comprises one or more 2' modified nucleotides, e.g., a 2'-deoxy, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O--N-methylacetamido (2'-O--NMA). In some embodiments, each nucleotide of the antisense strand is a modified nucleotide (e.g., a 2'-modified nucleotide). In some embodiments, the antisense strand comprises one or more phosphorodiamidate morpholinos. In some embodiments, the antisense strand is a phosphorodiamidate morpholino oligomer (PMO).

[000404] In some embodiments, antisense strand contains a phosphorothioate or other modified internucleotide linkage. In some embodiments, the antisense strand comprises phosphorothioate internucleoside linkages. In some embodiments, the antisense strand comprises phosphorothioate internucleoside linkages between at least two nucleotides. In some embodiments, the antisense strand comprises phosphorothioate internucleoside linkages between all nucleotides. For example, in some embodiments, the antisense strand comprises modified internucleotide linkages at the first, second, and/or (e.g., and) third internucleoside linkage at the 5' or 3' end of the siRNA molecule. In some embodiments, the modified internucleotide linkages are phosphorus-containing linkages. In some embodiments, phosphorus-containing linkages that may be used include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'; see US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5, 177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455, 233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563, 253; 5,571,799; 5,587,361; and 5,625,050.

[000405] Any of the modified chemistries or formats of the antisense strand described herein can be combined with each other. For example, one, two, three, four, five, or more different types of modifications can be included within the same antisense strand.

[000406] In some embodiments, the sense strand comprises one or more modified nucleotides (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more). In some embodiments, the sense strand comprises one or more modified nucleotides and/or (e.g., and) one or more modified internucleotide linkages. In some embodiments, the modified nucleotide is a modified sugar moiety (e.g. a 2' modified nucleotide). In some embodiments, the sense strand comprises one or more 2' modified nucleotides, e.g., a 2'-deoxy, 2'-fluoro (2'-F), 2'-O-methyl (2'-O-Me), 2'-O-methoxyethyl (2'-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O--N-methylacetamido (2'-O--NMA). In some embodiments, each nucleotide of the sense strand is a modified nucleotide (e.g., a 2'-modified nucleotide). In

some embodiments, the sense strand comprises one or more phosphorodiamidate morpholinos. In some embodiments, the antisense strand is a phosphorodiamidate morpholino oligomer (PMO). In some embodiments, the sense strand contains a phosphorothioate or other modified internucleotide linkage. In some embodiments, the sense strand comprises phosphorothioate internucleoside linkages. In some embodiments, the sense strand comprises phosphorothioate internucleoside linkages between at least two nucleotides. In some embodiments, the sense strand comprises phosphorothioate internucleoside linkages between all nucleotides. For example, in some embodiments, the sense strand comprises modified internucleotide linkages at the first, second, and/or (e.g., and) third internucleoside linkage at the 5' or 3' end of the sense strand.

[000407] In some embodiments, the modified internucleotide linkages are phosphorus-containing linkages. In some embodiments, phosphorus-containing linkages that may be used include, but are not limited to, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates comprising 3'alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates comprising 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'; see US patent nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5, 177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455, 233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563, 253; 5,571,799; 5,587,361; and 5,625,050.

[000408] Any of the modified chemistries or formats of the sense strand described herein can be combined with each other. For example, one, two, three, four, five, or more different types of modifications can be included within the same sense strand.

[000409] In some embodiments, the antisense or sense strand of the siRNA molecule comprises modifications that enhance or reduce RNA-induced silencing complex (RISC) loading. In some embodiments, the antisense strand of the siRNA molecule comprises modifications that enhance RISC loading. In some embodiments, the sense strand of the siRNA molecule comprises modifications that reduce RISC loading and reduce off-target effects. In some embodiments, the antisense strand of the siRNA molecule comprises a 2'-O-methoxyethyl (2'-MOE) modification. The addition of the 2'-O-methoxyethyl (2'-MOE) group at the cleavage site improves both the specificity and silencing activity of siRNAs by

facilitating the oriented RNA-induced silencing complex (RISC) loading of the modified strand, as described in Song et al., (2017) Mol Ther Nucleic Acids 9:242-250, incorporated herein by reference in its entirety. In some embodiments, the antisense strand of the siRNA molecule comprises a 2′-OMe-phosphorodithioate modification, which increases RISC loading as described in Wu et al., (2014) Nat Commun 5:3459, incorporated herein by reference in its entirety.

[000410] In some embodiments, the sense strand of the siRNA molecule comprises a 5'-morpholino, which reduces RISC loading of the sense strand and improves antisense strand selection and RNAi activity, as described in Kumar et al., (2019) Chem Commun (Camb) 55(35):5139-5142, incorporated herein by reference in its entirety. In some embodiments, the sense strand of the siRNA molecule is modified with a synthetic RNA-like high affinity nucleotide analogue, Locked Nucleic Acid (LNA), which reduces RISC loading of the sense strand and further enhances antisense strand incorporation into RISC, as described in Elman et al., (2005) Nucleic Acids Res. 33(1): 439-447, incorporated herein by reference in its entirety. In some embodiments, the sense strand of the siRNA molecule comprises a 5′ unlocked nucleic acic (UNA) modification, which reduce RISC loading of the sense strand and improve silencing potentcy of the antisense strand, as described in Snead et al., (2013) Mol Ther Nucleic Acids 2(7):e103, incorporated herein by reference in its entirety. In some embodimcnts, thc sense strand of the siRNA molecule comprises a 5-nitroindole modification, which descresed the RNAi potency of the sense strand and reduces off-targent effects as described in Zhang et al., (2012) Chembiochem 13(13):1940-1945, incorporated herein by reference in its entirety. In some embodiments, the sense strand comprises a 2'-O'methyl (2'-O-Me) modification, which reduces RISC loading and the off-target effects of the sense strand, as described in Zheng et al., FASEB (2013) 27(10): 4017-4026, incorporated herein by reference in its entirety. In some embodiments, the sense strand of the siRNA molecule is fully substituted with morpholino, 2'-MOE or 2'-O-Me residues, and are not recognized by RISC as described in Kole et al., (2012) Nature reviews. Drug Discovery 11(2):125-140, incorporated herein by reference in its entirety. In some embodiments the antisense strand of the siRNA molecule comprises a 2'-MOE modification and the sense strand comprises an 2'-O-Me modification (see e.g., Song et al., (2017) Mol Ther Nucleic Acids 9:242-250).In some embodiments at least one (e.g., at least 2, at least 3, at least 4, at least 5, at least 10) siRNA molecule is linked (e.g., covalently) to a muscle-targeting agent. In some embodiments, the muscle-targeting agent may comprise, or consist of, a nucleic acid (e.g., DNA or RNA), a peptide (e.g., an antibody), a lipid (e.g., a microvesicle), or a sugar moiety (e.g., a

polysaccharide). In some embodiments, the muscle-targeting agent is an antibody. In some embodiments, the muscle-targeting agent is an anti-transferrin receptor antibody (e.g., any one of the anti-TfR antibodies provided herein). In some embodiments, the muscle-targeting agent may be linked to the 5' end of the sense strand of the siRNA molecule. In some embodiments, the muscle-targeting agent may be linked to the 3' end of the sense strand of the siRNA molecule. In some embodiments, the muscle-targeting agent may be linked internally to the sense strand of the siRNA molecule. In some embodiments, the muscle-targeting agent may be linked to the 5' end of the antisense strand of the siRNA molecule. In some embodiments, the muscle-targeting agent may be linked to the 3' end of the antisense strand of the siRNA molecule. In some embodiments, the muscle-targeting agent may be linked internally to the antisense strand of the siRNA molecule.

k. microRNA (miRNAs)

[000411] In some embodiments, an oligonucleotide may be a microRNA (miRNA). MicroRNAs (referred to as "miRNAs") are small non-coding RNAs, belonging to a class of regulatory molecules that control gene expression by binding to complementary sites on a target RNA transcript. Typically, miRNAs are generated from large RNA precursors (termed pri-miRNAs) that are processed in the nucleus into approximately 70 nucleotide pre-miRNAs, which fold into imperfect stem-loop structures. These pre-miRNAs typically undergo an additional processing step within the cytoplasm where mature miRNAs of 18-25 nucleotides in length are excised from one side of the pre-miRNA hairpin by an RNase III enzyme, Dicer.

[000412] As used herein, miRNAs including pri-miRNA, pre-miRNA, mature miRNA or fragments of variants thereof that retain the biological activity of mature miRNA. In one embodiment, the size range of the miRNA can be from 21 nucleotides to 170 nucleotides. In one embodiment the size range of the miRNA is from 70 to 170 nucleotides in length. In another embodiment, mature miRNAs of from 21 to 25 nucleotides in length can be used.

l. Aptamers

[000413] In some embodiments, oligonucleotides provided herein may be in the form of aptamers. Generally, in the context of molecular payloads, aptamer is any nucleic acid that binds specifically to a target, such as a small molecule, protein, nucleic acid in a cell. In some embodiments, the aptamer is a DNA aptamer or an RNA aptamer. In some embodiments, a nucleic acid aptamer is a single-stranded DNA or RNA (ssDNA or ssRNA). It is to be understood that a single-stranded nucleic acid aptamer may form helices and/or (e.g., and) loop structures. The nucleic acid that forms the nucleic acid aptamer may comprise naturally

occurring nucleotides, modified nucleotides, naturally occurring nucleotides with hydrocarbon linkers (e.g., an alkylene) or a polyether linker (e.g., a PEG linker) inserted between one or more nucleotides, modified nucleotides with hydrocarbon or PEG linkers inserted between one or more nucleotides, or a combination of thereof. Exemplary publications and patents describing aptamers and method of producing aptamers include, e.g., Lorsch and Szostak, 1996; Jayasena, 1999; U.S. Pat. Nos. 5,270,163; 5,567,588; 5,650,275; 5,670,637; 5,683,867; 5,696,249; 5,789,157; 5,843,653; 5,864,026; 5,989,823; 6,569,630; 8,318,438 and PCT application WO 99/31275, each incorporated herein by reference.

m. Ribozymes

[000414] In some embodiments, oligonucleotides provided herein may be in the form of a ribozyme. A ribozyme (ribonucleic acid enzyme) is a molecule, typically an RNA molecule, that is capable of performing specific biochemical reactions, similar to the action of protein enzymes. Ribozymes are molecules with catalytic activities including the ability to cleave at specific phosphodiester linkages in RNA molecules to which they have hybridized, such as mRNAs, RNA-containing substrates, lncRNAs, and ribozymes, themselves.

[000415] Ribozymes may assume one of several physical structures, one of which is called a "hammerhead." A hammerhead ribozyme is composed of a catalytic core containing nine conserved bases, a double-stranded stem and loop structure (stem-loop II), and two regions complementary to the target RNA flanking regions the catalytic core. The flanking regions enable the ribozyme to bind to the target RNA specifically by forming double-stranded stems I and III. Cleavage occurs in cis (i.e., cleavage of the same RNA molecule that contains the hammerhead motif) or in trans (cleavage of an RNA substrate other than that containing the ribozyme) next to a specific ribonucleotide triplet by a transesterification reaction from a 3', 5'-phosphate diester to a 2', 3'-cyclic phosphate diester. Without wishing to be bound by theory, it is believed that this catalytic activity requires the presence of specific, highly conserved sequences in the catalytic region of the ribozyme.

[000416] Modifications in ribozyme structure have also included the substitution or replacement of various non-core portions of the molecule with non-nucleotidic molecules. For example, Benseler et al. (J. Am. Chem. Soc. (1993) 115:8483-8484) disclosed hammerhead-like molecules in which two of the base pairs of stem II, and all four of the nucleotides of loop II were replaced with non-nucleoside linkers based on hexaethylene glycol, propanediol, bis(triethylene glycol) phosphate, tris(propanediol)bisphosphate, or bis(propanediol) phosphate. Ma et al. (Biochem. (1993) 32:1751-1758; Nucleic Acids Res. (1993) 21:2585-2589) replaced the six nucleotide loop of the TAR ribozyme hairpin with non-nucleotidic,

ethylene glycol-related linkers. Thomson et al. (Nucleic Acids Res. (1993) 21:5600-5603) replaced loop II with linear, non-nucleotidic linkers of 13, 17, and 19 atoms in length.

[000417] Ribozyme oligonucleotides can be prepared using well known methods (see, e.g., PCT Publications WO9118624; WO9413688; WO9201806; and WO 92/07065; and U.S. Patents 5436143 and 5650502) or can be purchased from commercial sources (e.g., US Biochemicals) and, if desired, can incorporate nucleotide analogs to increase the resistance of the oligonucleotide to degradation by nucleases in a cell. The ribozyme may be synthesized in any known manner, e.g., by use of a commercially available synthesizer produced, e.g., by Applied Biosystems, Inc. or Milligen. The ribozyme may also be produced in recombinant vectors by conventional means. See, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (Current edition). The ribozyme RNA sequences maybe synthesized conventionally, for example, by using RNA polymerases such as T7 or SP6.

n. Guide Nucleic Acids

[000418] In some embodiments, oligonucleotides are guide nucleic acid, e.g., guide RNA (gRNA) molecules. Generally, a guide RNA is a short synthetic RNA composed of (1) a scaffold sequence that binds to a nucleic acid programmable DNA binding protein (napDNAbp), such as Cas9, and (2) a nucleotide spacer portion that defines the DNA target sequence (e.g., genomic DNA target) to which the gRNA binds in order to bring the nucleic acid programmable DNA binding protein in proximity to the DNA target sequence. In some embodiments, the napDNAbp is a nucleic acid-programmable protein that forms a complex with (e.g., binds or associates with) one or more RNA(s) that targets the nucleic acid-programmable protein to a target DNA sequence (e.g., a target genomic DNA sequence). In some embodiments, a nucleic acid -programmable nuclease, when in a complex with an RNA, may be referred to as a nuclease:RNA complex. Guide RNAs can exist as a complex of two or more RNAs, or as a single RNA molecule.

[000419] Guide RNAs (gRNAs) that exist as a single RNA molecule may be referred to as single-guide RNAs (sgRNAs), though gRNA is also used to refer to guide RNAs that exist as either single molecules or as a complex of two or more molecules. Typically, gRNAs that exist as a single RNA species comprise two domains: (1) a domain that shares homology to a target nucleic acid (i.e., directs binding of a Cas9 complex to the target); and (2) a domain that binds a Cas9 protein. In some embodiments, domain (2) corresponds to a sequence known as a tracrRNA and comprises a stem-loop structure. In some embodiments, domain (2) is identical or homologous to a tracrRNA as provided in Jinek et al., Science 337:816-821 (2012), the entire contents of which is incorporated herein by reference.

[000420] In some embodiments, a gRNA comprises two or more of domains (1) and (2), and may be referred to as an extended gRNA. For example, an extended gRNA will bind two or more Cas9 proteins and bind a target nucleic acid at two or more distinct regions, as described herein. The gRNA comprises a nucleotide sequence that complements a target site, which mediates binding of the nuclease/RNA complex to said target site, providing the sequence specificity of the nuclease:RNA complex. In some embodiments, the RNA-programmable nuclease is the (CRISPR-associated system) Cas9 endonuclease, for example, Cas9 (Csn1) from Streptococcus pyogenes (see, e.g., "Complete genome sequence of an M1 strain of Streptococcus pyogenes." Ferretti J.J., McShan W.M., Ajdic D.J., Savic D.J., Savic G., Lyon K., Primeaux C., Sezate S., Suvorov A.N., Kenton S., Lai H.S., Lin S.P., Qian Y., Jia H.G., Najar F.Z., Ren Q., Zhu H., Song L., White J., Yuan X., Clifton S.W., Roe B.A., McLaughlin R.E., Proc. Natl. Acad. Sci. U.S.A. 98:4658-4663 (2001); "CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III." Deltcheva E., Chylinski K., Sharma C.M., Gonzales K., Chao Y., Pirzada Z.A., Eckert M.R., Vogel J., Charpentier E., Nature 471:602-607 (2011); and "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." Jinek M., Chylinski K., Fonfara I., Hauer M., Doudna J.A., Charpentier E. Science 337:816-821 (2012), the entire contents of each of which are incorporated herein by reference.

o. Multimers

[000421] In some embodiments, molecular payloads may comprise multimers (e.g., concatemers) of 2 or more oligonucleotides connected by a linker. In this way, in some embodiments, the oligonucleotide loading of a complex can be increased beyond the available linking sites on a targeting agent (e.g., available thiol sites on an antibody) or otherwise tuned to achieve a particular payload loading content. Oligonucleotides in a multimer can be the same or different (e.g., targeting different genes or different sites on the same gene or products thereof).

[000422] In some embodiments, multimers comprise 2 or more oligonucleotides linked together by a cleavable linker. However, in some embodiments, multimers comprise 2 or more oligonucleotides linked together by a non-cleavable linker. In some embodiments, a multimer comprises 2, 3, 4, 5, 6, 7, 8, 9, 10 or more oligonucleotides linked together. In some embodiments, a multimer comprises 2 to 5, 2 to 10 or 4 to 20 oligonucleotides linked together.

[000423] In some embodiments, a multimer comprises 2 or more oligonucleotides linked end-to-end (in a linear arrangement). In some embodiments, a multimer comprises 2 or more oligonucleotides linked end-to-end via a oligonucleotide based linker (e.g., poly-dT linker, an

abasic linker). In some embodiments, a multimer comprises a 5' end of one oligonucleotide linked to a 3' end of another oligonucleotide. In some embodiments, a multimer comprises a 3' end of one oligonucleotide linked to a 3' end of another oligonucleotide. In some embodiments, a multimer comprises a 5' end of one oligonucleotide linked to a 5' end of another oligonucleotide. Still, in some embodiments, multimers can comprise a branched structure comprising multiple oligonucleotides linked together by a branching linker.

[000424] Further examples of multimers that may be used in the complexes provided herein are disclosed, for example, in US Patent Application Number 2015/0315588 A1, entitled *Methods of delivering multiple targeting oligonucleotides to a cell using cleavable linkers*, which was published on November 5, 2015; US Patent Application Number 2015/0247141 A1, entitled *Multimeric Oligonucleotide Compounds*, which was published on September 3, 2015, US Patent Application Number US 2011/0158937 A1, entitled *Immunostimulatory Oligonucleotide Multimers*, which was published on June 30, 2011; and US Patent Number 5,693,773, entitled *Triplex-Forming Antisense Oligonucleotides Having Abasic Linkers Targeting Nucleic Acids Comprising Mixed Sequences Of Purines And Pyrimidines,* which issued on December 2, 1997, the contents of each of which are incorporated herein by reference in their entireties.

ii. Small Molecules:

[000425] Any suitable small molecule may be used as a molecular payload, as described herein. In some embodiments, the small molecule enhances exon skipping of DMD mutant sequences. In some embodiments, the small molecule is as described in US Patent Application Publication US20140080896A1, published March 20, 2014, entitled "IDENTIFICATION OF SMALL MOLECULES THAT FACILITATE THERAPEUTIC EXON SKIPPING". Further examples of small molecule payloads are provided in U.S. Patent No. 9,982,260, issued May 29, 2018, entitled "Identification of structurally similar small molecules that enhance therapeutic exon skipping". For example, in some embodiments, the small molecule is an enhancer of exon skipping such as perphenazine, flupentixol, zuclopenthixol or corynanthine. In some embodiments, a small molecule enhancer of exon skipping inhibits the ryanodine receptor or calmodulin. In some embodiments, the small molecule is an H-Ras pathway inhibitor such as manumycin A. In some embodiments, the small molecule is a suppressor of stop codons and desensitizes ribosomes to premature stop codons. In some embodiments, the small molecule is ataluren, as described in McElroy S.P. et al. "A Lack of Premature Termination Codon Read Through Efficacy of PTC124 (Ataluren) in a Diverse Array of Reporter Assays." PLOS Biology, published June 25, 2013. In some embodiments, the small

molecule is a corticosteroid, *e.g.,* as described in Manzur, A.Y. et al. "Glucocorticoid corticosteroids for Duchenne muscular dystrophy". Cochrane Database Syst Rev. 2004;(2):CD003725. In some embodiments, the small molecule upregulates the expression and/or (e.g., and) activity of genes that can replace the function of dystrophin, such as utrophin. In some embodiments, a utrophin modulator is as described in International Publication No. WO2007091106, published August 16, 2007, entitled "TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY" and/or (e.g., and) International Publication No. WO/2017/168151, published October 5, 2017, entitled "COMPOSITION FOR THE TREATMENT OF DUCHENNE MUSCULAR DYSTROPHY".

iii. Peptides/Proteins

[000426] Any suitable peptide or protein may be used as a molecular payload, as described herein. In some embodiments, a protein is an enzyme. In some embodiments, peptides or proteins may be produced, synthesized, and/or (e.g., and) derivatized using several methodologies, e.g. phage displayed peptide libraries, one-bead one-compound peptide libraries, or positional scanning synthetic peptide combinatorial libraries. Exemplary methodologies have been characterized in the art and are incorporated by reference (Gray, B.P. and Brown, K.C. "Combinatorial Peptide Libraries: Mining for Cell-Binding Peptides" Chem Rev. 2014, 114:2, 1020–1081.; Samoylova, T.I. and Smith, B.F. "Elucidation of muscle-binding peptides by phage display screening." Muscle Nerve, 1999, 22:4. 460-6.).

[000427] In some embodiments, a peptide may facilitate exon skipping in an mRNA expressed from a mutated DMD allele. In some embodiments, a peptide may promote the expression of functional dystrophin and/or (e.g., and) the expression of a protein capable of functioning in place of dystrophin. In some embodiments, payload is a protein that is a functional fragment of dystrophin, *e.g.* an amino acid segment of a functional dystrophin protein.

[000428] In some embodiments, the peptide or protein comprises at least one zinc finger.

[000429] In some embodiments, the peptide or protein may comprise about 2-25 amino acids, about 2-20 amino acids, about 2-15 amino acids, about 2-10 amino acids, or about 2-5 amino acids. The peptide or protein may comprise naturally-occurring amino acids, e.g. cysteine, alanine, or non-naturally-occurring or modified amino acids. Non-naturally occurring amino acids include β-amino acids, homo-amino acids, proline derivatives, 3-substituted alanine derivatives, linear core amino acids, N-methyl amino acids, and others known in the art. In some embodiments, the peptide may be linear; in other embodiments, the peptide may be cyclic, e.g. bicyclic.

iv. Nucleic Acid Constructs

[000430] Any suitable gene expression construct may be used as a molecular payload, as described herein. In some embodiments, a gene expression construct may be a vector or a cDNA fragment. In some embodiments, a gene expression construct may be messenger RNA (mRNA). In some embodiments, a mRNA used herein may be a modified mRNA, e.g., as described in US Patent 8,710,200, issued on April 24, 2014, entitled "*Engineered nucleic acids encoding a modified erythropoietin and their expression*". In some embodiments, a mRNA may comprise a 5′ methyl cap. In some embodiments, a mRNA may comprise a polyA tail, optionally of up to 160 nucleotides in length. A gene expression construct may encode a sequence of a dystrophin protein, a dystrophin fragment, a mini-dystrophin, a utrophin protein, or any protein that shares a common function with dystrophin. In some embodiments, the gene expression construct may be expressed, e.g., overexpressed, within the nucleus of a muscle cell. In some embodiments, the gene expression constructs encodes a protein that comprises at least one zinc finger. In some embodiments, the gene expression construct encodes a protein that promotes the expression of dystrophin or a protein that shares function with dystrophin, *e.g.*, utrophin. In some embodiments, the gene expression construct encodes a gene editing enzyme. In some embodiments, the gene expression construct is as described in U.S. Patent Application Publication US20170368198A1, published December 28, 2017, entitled "Optimized mini-dystrophin genes and expression cassettes and their use"; Duan D. "Myodys, a full-length dystrophin plasmid vector for Duchenne and Becker muscular dystrophy gene therapy." Curr Opin Mol Ther 2008;10:86–94; and expression cassettes disclosed in Tang, Y. et al., "AAV-directed muscular dystrophy gene therapy" Expert Opin Biol Ther. 2010 Mar;10(3):395-408; the contents of each of which are incorporated herein by reference in their entireties.

C. Linkers

[000431] Complexes described herein generally comprise a linker that connects any one of the anti-TfR antibodies described herein to a molecular payload. A linker comprises at least one covalent bond. In some embodiments, a linker may be a single bond, e.g., a disulfide bond or disulfide bridge, that connects an anti-TfR antibody to a molecular payload. However, in some embodiments, a linker may connect any one of the anti-TfR antibodies described herein to a molecular payload through multiple covalent bonds. In some embodiments, a linker may be a cleavable linker. However, in some embodiments, a linker may be a non-cleavable linker. A linker is generally stable *in vitro* and *in vivo*, and may be stable in certain cellular environments. Additionally, generally a linker does not negatively impact the functional

properties of either the anti-TfR antibody or the molecular payload. Examples and methods of synthesis of linkers are known in the art (see, e.g. Kline, T. et al. "Methods to Make Homogenous Antibody Drug Conjugates." Pharmaceutical Research, 2015, 32:11, 3480–3493.; Jain, N. et al. "Current ADC Linker Chemistry" Pharm Res. 2015, 32:11, 3526–3540.; McCombs, J.R. and Owen, S.C. "Antibody Drug Conjugates: Design and Selection of Linker, Payload and Conjugation Chemistry" AAPS J. 2015, 17:2, 339–351.).

[000432] A precursor to a linker typically will contain two different reactive species that allow for attachment to both the anti-TfR antibody and a molecular payload. In some embodiments, the two different reactive species may be a nucleophile and/or (e.g., and) an electrophile. In some embodiments, a linker is connected to an anti-TfR antibody via conjugation to a lysine residue or a cysteine residue of the anti-TfR antibody. In some embodiments, a linker is connected to a cysteine residue of an anti-TfR antibody via a maleimide-containing linker, wherein optionally the maleimide-containing linker comprises a maleimidocaproyl or maleimidomethyl cyclohexane-1-carboxylate group. In some embodiments, a linker is connected to a cysteine residue of an anti-TfR antibody or thiol functionalized molecular payload via a 3-arylpropionitrile functional group. In some embodiments, a linker is connected to a lysine residue of an anti-TfR antibody. In some embodiments, a linker is connected to an anti-TfR antibody and/or (e.g., and) a molecular payload via an amide bond, a carbamate bond, a hydrazide, a trizaole, a thioether, or a disulfide bond.

i. Cleavable Linkers

[000433] A cleavable linker may be a protease-sensitive linker, a pH-sensitive linker, or a glutathione-sensitive linker. These linkers are generally cleavable only intracellularly and are preferably stable in extracellular environments, e.g. extracellular to a muscle cell.

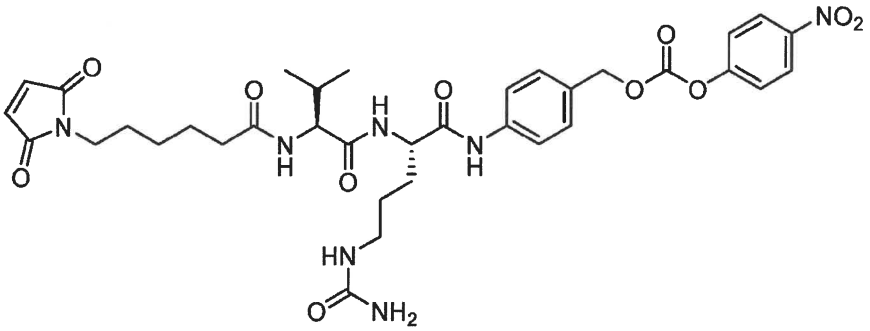
[000434] Protease-sensitive linkers are cleavable by protease enzymatic activity. These linkers typically comprise peptide sequences and may be 2-10 amino acids, about 2-5 amino acids, about 5-10 amino acids, about 10 amino acids, about 5 amino acids, about 3 amino acids, or about 2 amino acids in length. In some embodiments, a peptide sequence may comprise naturally-occurring amino acids, e.g. cysteine, alanine, or non-naturally-occurring or modified amino acids. Non-naturally occurring amino acids include β-amino acids, homo-amino acids, proline derivatives, 3-substituted alanine derivatives, linear core amino acids, N-methyl amino acids, and others known in the art. In some embodiments, a protease-sensitive linker comprises a valine-citrulline or alanine-citrulline dipeptide sequence. In some

embodiments, a protease-sensitive linker can be cleaved by a lysosomal protease, e.g. cathepsin B, and/or (e.g., and) an endosomal protease.

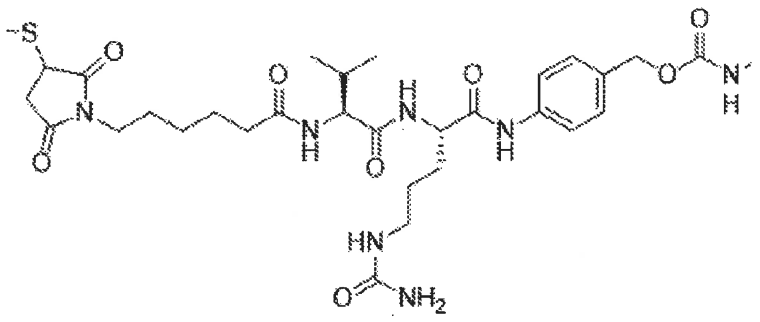
[000435] A pH-sensitive linker is a covalent linkage that readily degrades in high or low pH environments. In some embodiments, a pH-sensitive linker may be cleaved at a pH in a range of 4 to 6. In some embodiments, a pH-sensitive linker comprises a hydrazone or cyclic acetal. In some embodiments, a pH-sensitive linker is cleaved within an endosome or a lysosome.

[000436] In some embodiments, a glutathione-sensitive linker comprises a disulfide moiety. In some embodiments, a glutathione-sensitive linker is cleaved by a disulfide exchange reaction with a glutathione species inside a cell. In some embodiments, the disulfide moiety further comprises at least one amino acid, e.g. a cysteine residue.

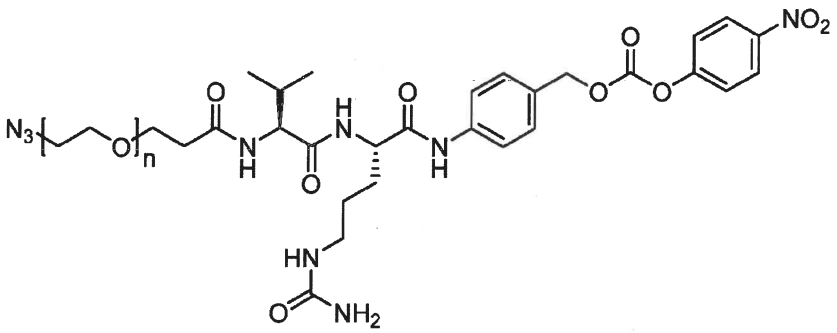
[000437] In some embodiments, the linker is a Val-cit linker (e.g., as described in US Patent 6,214,345, incorporated herein by reference). In some embodiments, before conjugation, the val-cit linker has a structure of:



[000438] In some embodiments, after conjugation, the val-cit linker has a structure of:

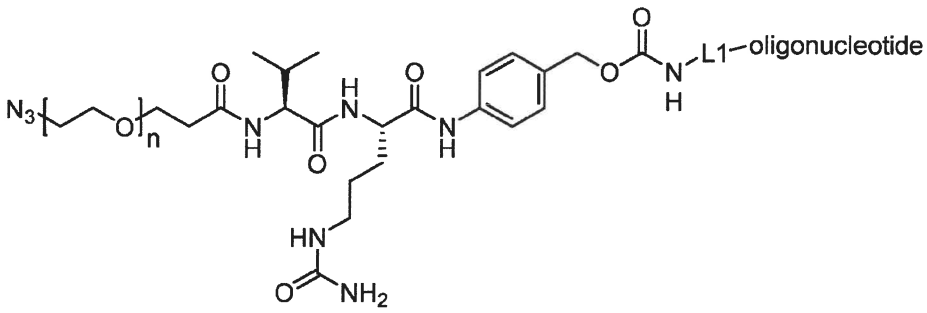


[000439] In some embodiments, the Val-cit linker is attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation). In some embodiments, before click chemistry conjugation, the val-cit linker attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation) has the structure of:



wherein n is any number from 0-10. In some embodiments, n is 3.

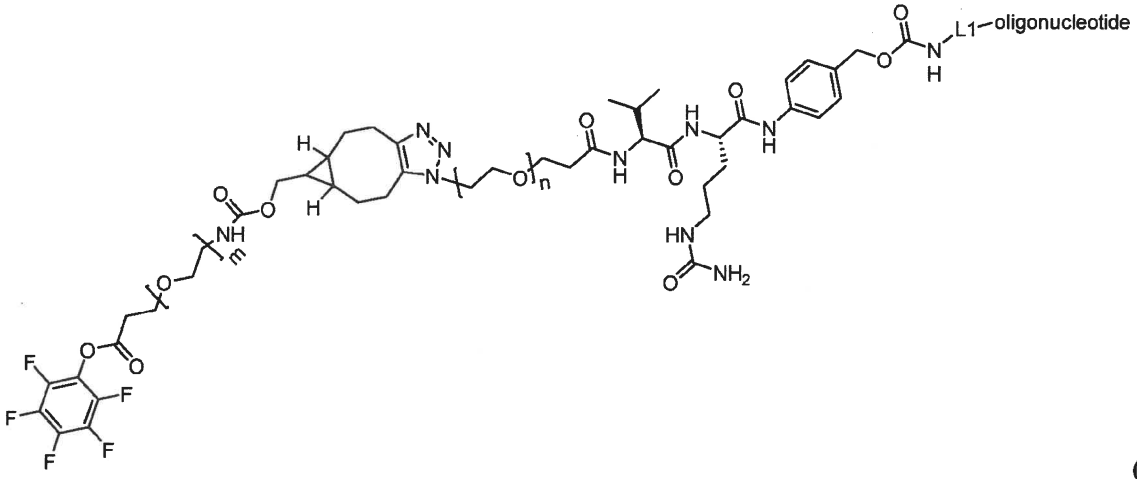
[000440] In some embodiments, the val-cit linker attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation) is conjugated (e.g., via a different chemical moiety) to a molecular payload (e.g., an oligonucleotide). In some embodiments, the val-cit linker attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation) and conjugated to a molecular payload (e.g., an oligonucleotide) has the structure of (before click chemistry conjugation):



(A)

wherein n is any number from 0-10. In some embodiments, n is 3.

[000441] In some embodiments, after conjugation to a molecular payload (e.g., an oligonucleotide), the val-cit linker has a structure of:



(B)

wherein n is any number from 0-10, and wherein m is any number from 0-10. In some embodiments, n is 3 and m is 4.

ii. Non-Cleavable Linkers

[000442] In some embodiments, non-cleavable linkers may be used. Generally, a non-cleavable linker cannot be readily degraded in a cellular or physiological environment. In some embodiments, a non-cleavable linker comprises an optionally substituted alkyl group, wherein the substitutions may include halogens, hydroxyl groups, oxygen species, and other common substitutions. In some embodiments, a linker may comprise an optionally substituted alkyl, an optionally substituted alkylene, an optionally substituted arylene, a heteroarylene, a peptide sequence comprising at least one non-natural amino acid, a truncated glycan, a sugar or sugars that cannot be enzymatically degraded, an azide, an alkyne-azide, a peptide sequence comprising a LPXT sequence, a thioether, a biotin, a biphenyl, repeating units of polyethylene glycol or equivalent compounds, acid esters, acid amides, sulfamides, and/or (e.g., and) an alkoxy-amine linker. In some embodiments, sortase-mediated ligation will be utilized to covalently link an anti-TfR antibody comprising a LPXT sequence to a molecular payload comprising a $(G)_n$ sequence (see, e.g. Proft T. Sortase-mediated protein ligation: an emerging biotechnology tool for protein modification and immobilization. Biotechnol Lett. 2010, 32(1):1-10.).

[000443] In some embodiments, a linker may comprise a substituted alkylene, an optionally substituted alkenylene, an optionally substituted alkynylene, an optionally substituted cycloalkylene, an optionally substituted cycloalkenylene, an optionally substituted arylene, an optionally substituted heteroarylene further comprising at least one heteroatom selected from N, O, and S,; an optionally substituted heterocyclylene further comprising at least one heteroatom selected from N, O, and S,; an imino, an optionally substituted nitrogen species, an optionally substituted oxygen species O, an optionally substituted sulfur species, or a poly(alkylene oxide), e.g. polyethylene oxide or polypropylene oxide.

iii. Linker conjugation

[000444] In some embodiments, a linker is connected to an anti-TfR antibody and/or (e.g., and) molecular payload via a phosphate, thioether, ether, carbon-carbon, carbamate, or amide bond. In some embodiments, a linker is connected to an oligonucleotide through a phosphate or phosphorothioate group, e.g. a terminal phosphate of an oligonucleotide backbone. In some embodiments, a linker is connected to an anti-TfR antibody, through a lysine or cysteine residue present on the anti-TfR antibody.

[000445] In some embodiments, a linker is connected to an anti-TfR antibody and/or (e.g., and) molecular payload by a cycloaddition reaction between an azide and an alkyne to

form a triazole, wherein the azide and the alkyne may be located on the anti-TfR antibody, molecular payload, or the linker. In some embodiments, an alkyne may be a cyclic alkyne, *e.g.*, a cyclooctyne. In some embodiments, an alkyne may be bicyclononyne (also known as bicyclo[6.1.0]nonyne or BCN) or substituted bicyclononyne. In some embodiments, a cyclooctane is as described in International Patent Application Publication WO2011136645, published on November 3, 2011, entitled, "*Fused Cyclooctyne Compounds And Their Use In Metal-free Click Reactions*". In some embodiments, an azide may be a sugar or carbohydrate molecule that comprises an azide. In some embodiments, an azide may be 6-azido-6-deoxygalactose or 6-azido-N-acetylgalactosamine. In some embodiments, a sugar or carbohydrate molecule that comprises an azide is as described in International Patent Application Publication WO2016170186, published on October 27, 2016, entitled, "*Process For The Modification Of A Glycoprotein Using A Glycosyltransferase That Is Or Is Derived From A β(1,4)-N-Acetylgalactosaminyltransferase*". In some embodiments, a cycloaddition reaction between an azide and an alkyne to form a triazole, wherein the azide and the alkyne may be located on the anti-TfR antibody, molecular payload, or the linker is as described in International Patent Application Publication WO2014065661, published on May 1, 2014, entitled, "*Modified antibody, antibody-conjugate and process for the preparation thereof*"; or International Patent Application Publication WO2016170186, published on October 27, 2016, entitled, "*Process For The Modification Of A Glycoprotein Using A Glycosyltransferase That Is Or Is Derived From A β(1,4)-N-Acetylgalactosaminyltransferase*".

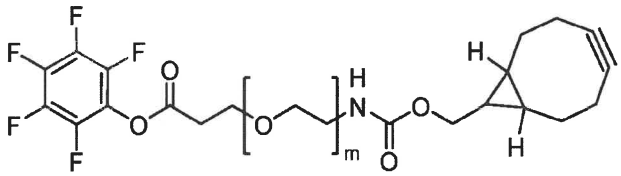
[000446] In some embodiments, a linker further comprises a spacer, *e.g.*, a polyethylene glycol spacer or an acyl/carbomoyl sulfamide spacer, *e.g.*, a HydraSpace™ spacer. In some embodiments, a spacer is as described in Verkade, J.M.M. et al., "*A Polar Sulfamide Spacer Significantly Enhances the Manufacturability, Stability, and Therapeutic Index of Antibody-Drug Conjugates*", Antibodies, 2018, 7, 12.

[000447] In some embodiments, a linker is connected to an anti-TfR antibody and/or (e.g., and) molecular payload by the Diels-Alder reaction between a dienophile and a diene/hetero-diene, wherein the dienophile and the dienc/hetero-diene may be located on the anti-TfR antibody, molecular payload, or the linker. In some embodiments a linker is connected to an anti-TfR antibody and/or (e.g., and) molecular payload by other pericyclic reactions, e.g. ene reaction. In some embodiments, a linker is connected to an anti-TfR antibody and/or (e.g., and) molecular payload by an amide, thioamide, or sulfonamide bond reaction. In some embodiments, a linker is connected to an anti-TfR antibody and/or (e.g., and) molecular payload by a condensation reaction to form an oxime, hydrazone, or

semicarbazide group existing between the linker and the anti-TfR antibody and/or (e.g., and) molecular payload.

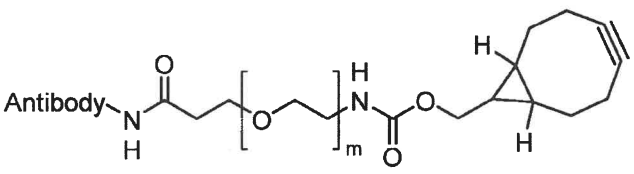
[000448] In some embodiments, a linker is connected to an anti-TfR antibody and/or (e.g., and) molecular payload by a conjugate addition reactions between a nucleophile, e.g. an amine or a hydroxyl group, carbonate, and an electrophile, e.g. a carboxylic acid or an aldehyde. In some embodiments, a nucleophile may exist on a linker and an electrophile may exist on an anti-TfR antibody or molecular payload prior to a reaction between a linker and an anti-TfR antibody or molecular payload. In some embodiments, an electrophile may exist on a linker and a nucleophile may exist on an anti-TfR antibody or molecular payload prior to a reaction between a linker and an anti-TfR antibody or molecular payload. In some embodiments, an electrophile may be an azide, pentafluorophenyl, a silicon centers, a carbonyl, a carboxylic acid, an anhydride, an isocyanate, a thioisocyanate, a succinimidyl ester, a sulfosuccinimidyl ester, a maleimide, an alkyl halide, an alkyl pseudohalide, an epoxide, an episulfide, an aziridine, an aryl, an activated phosphorus center, and/or (e.g., and) an activated sulfur center. In some embodiments, a nucleophile may be an optionally substituted alkene, an optionally substituted alkyne, an optionally substituted aryl, an optionally substituted heterocyclyl, a hydroxyl group, an amino group, an alkylamino group, an anilido group, or a thiol group.

[000449] In some embodiments, the val-cit linker attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation) is conjugated to the anti-TfR antibody by a structure of:



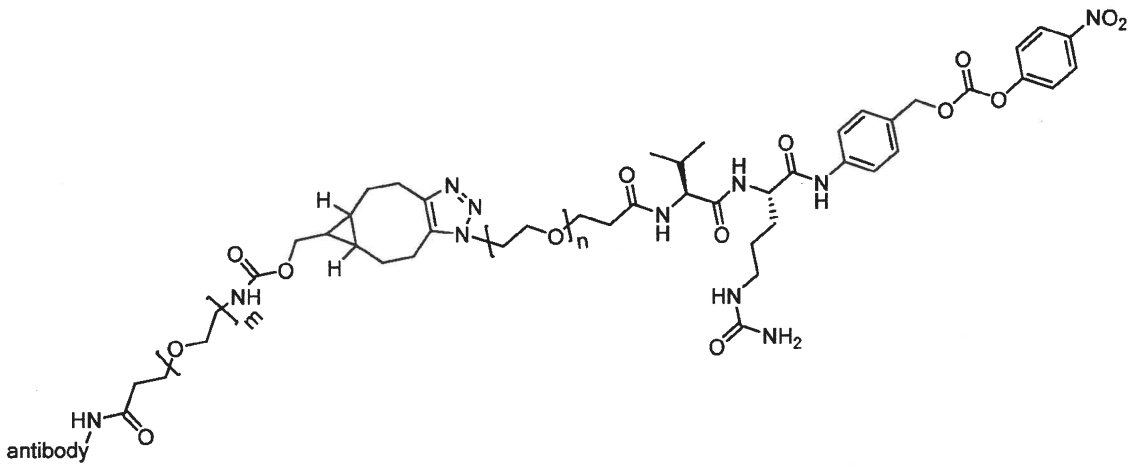
wherein m is any number from 0-10. In some embodiments, m is 4.

[000450] In some embodiments, the val-cit linker attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation) is conjugated to an anti-TfR antibody having a structure of:



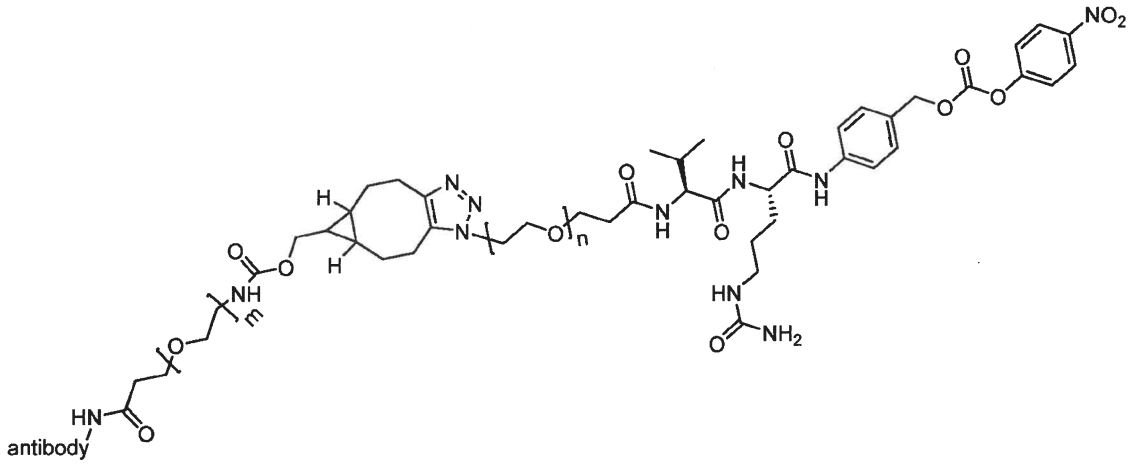
wherein m is any number from 0-10. In some embodiments, m is 4.

[000451] In some embodiments, the val-cit linker attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation) and conjugated to an anti-TfR antibody has a structure of:



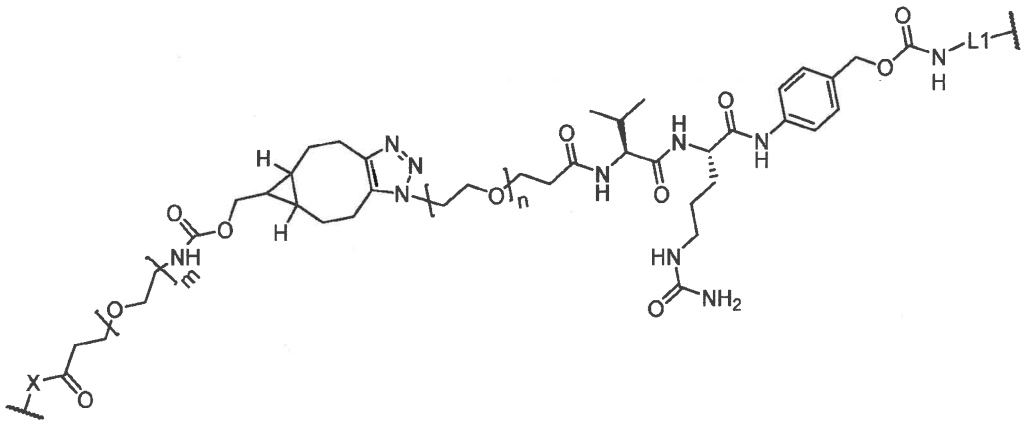
wherein n is any number from 0-10, wherein m is any number from 0-10. In some embodiments, n is 3 and/or (e.g., and) m is 4.

[000452] In some embodiments, the val-cit linker used to covalently link an anti-TfR antibody and a molecular payload (e.g., an oligonucleotide) has a structure of:



wherein n is 3 and m is 4.

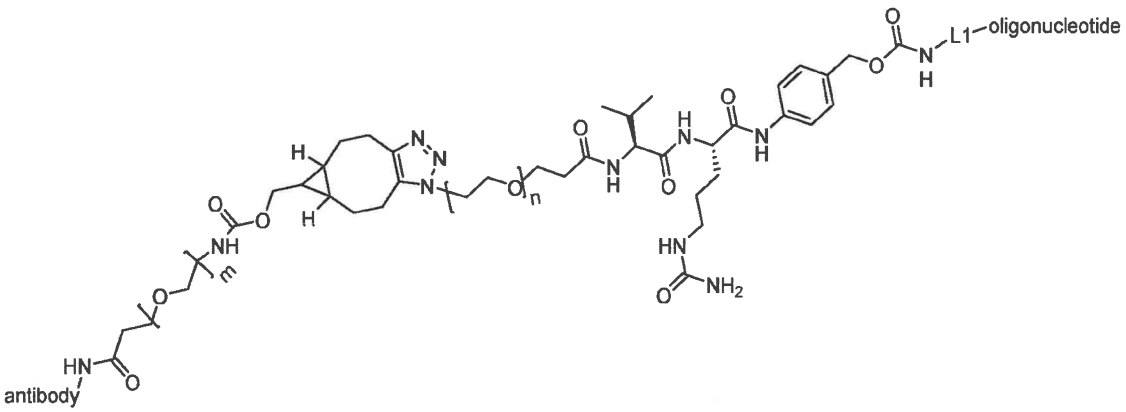
[000453] In some embodiments, the val-cit linker that links the antibody and the molecular payload has a structure of:



(C)

wherein n is any number from 0-10, wherein m is any number from 0-10. In some embodiments, n is 3 and/or (e.g., and) m is 4. In some embodiments, n is 3 and/or (e.g., and) m is 4. In some embodiments, X is NH (e.g., NH from an amine group of a lysine), S (e.g., S from a thiol group of a cysteine), or O (e.g., O from a hydroxyl group of a serine, threonine, or tyrosine) of the antibody.

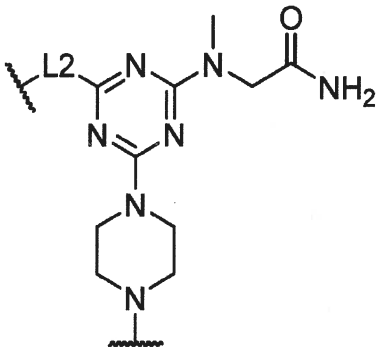
[000454] In some embodiments, the complex described herein has a structure of:



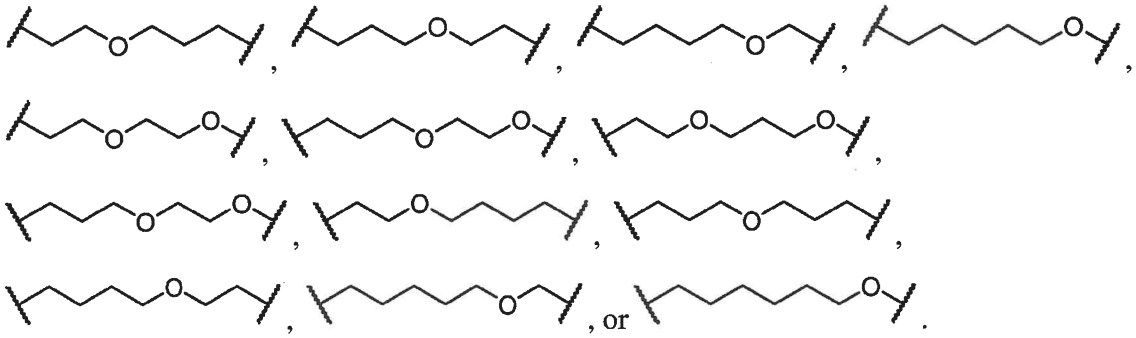
(D)

wherein n is any number from 0-10, wherein m is any number from 0-10. In some embodiments, n is 3 and/or (e.g., and) m is 4.

[000455] In structures formula (A), (B), (C), and (D), L1, in some embodiments, is a spacer that is a substituted or unsubstituted aliphatic, substituted or unsubstituted heteroaliphatic, substituted or unsubstituted carbocyclylene, substituted or unsubstituted heterocyclylene, substituted or unsubstituted arylene, substituted or unsubstituted heteroarylene, -O-, -N(R^A)-, -S-, -C(=O)-, -C(=O)O-, -C(=O)NR^A-, -NR^AC(=O)-, -NR^AC(=O)R^A-, -C(=O)R^A-, -NR^AC(=O)O-, -NR^AC(=O)N(R^A)-, -OC(=O)-, -OC(=O)O-, -OC(=O)N(R^A)-, -$S(O)_2$NR^A-, -N$R^A$$S(O)_2$-, or a combination thereof. In some embodiments, L1 is



wherein the piperazine moiety links to the oligonucleotide, wherein L2 is


O
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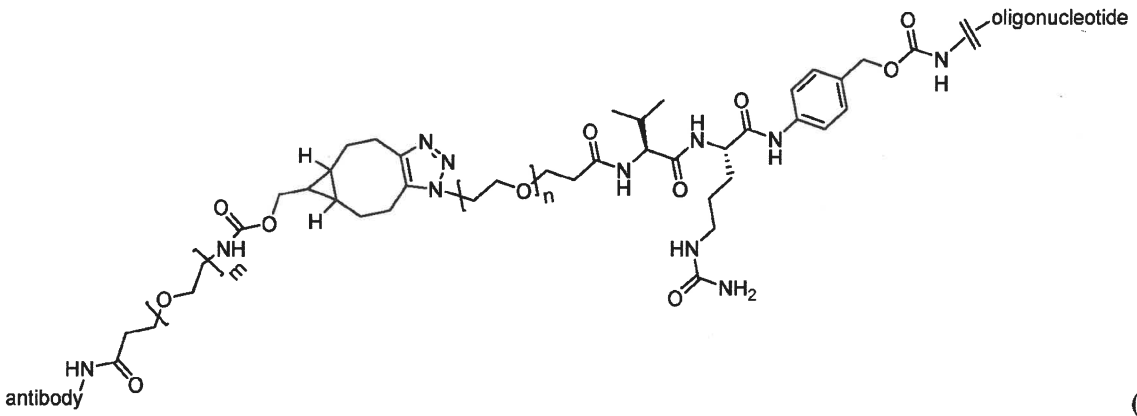


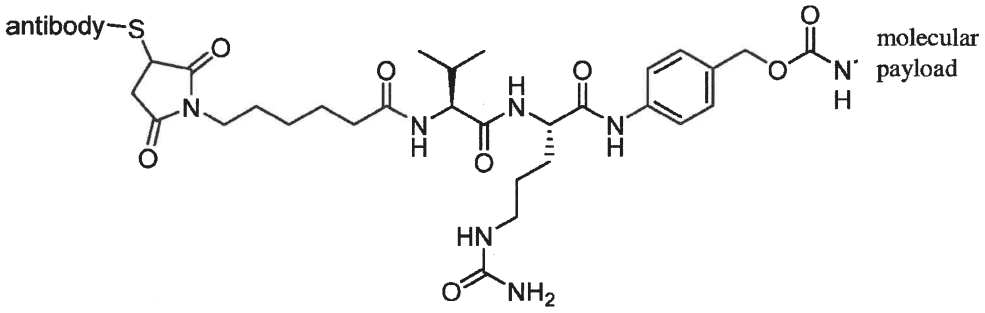
(E)

wherein n is 0-15 (e.g., 3) and m is 0-15 (e.g., 4).

C. Examples of Antibody-Molecular Payload Complexes

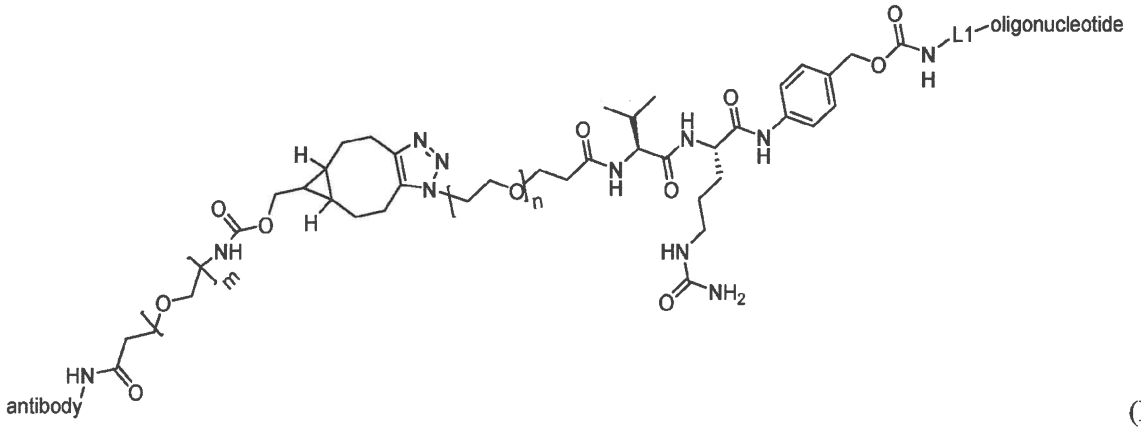
[000461] Further provided herein are non-limiting examples of complexes comprising any one the anti-TfR antibodies described herein covalently linked to any of the molecular payloads (e.g., an oligonucleotide) described herein. In some embodiments, the anti-TfR antibody (e.g., any one of the anti-TfR antibodies provided in Table 2) is covalently linked to a molecular payload (e.g., an oligonucleotide) via a linker. Any of the linkers described herein may be used. In some embodiments, if the molecular payload is an oligonucleotide, the linker is linked to the 5′ end, the 3′ end, or internally of the oligonucleotide. In some embodiments, the linker is linked to the anti-TfR antibody via a thiol-reactive linkage (e.g., via a cysteine in the anti-TfR antibody). In some embodiments, the linker (e.g., a Val-cit linker) is linked to the antibody (e.g., an anti-TfR antibody described herein) via an amine group (e.g., via a lysine in the antibody). In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046).

[000462] An example of a structure of a complex comprising an anti-TfR antibody covalently linked to a molecular payload via a Val-cit linker is provided below:



wherein the linker is linked to the antibody via a thiol-reactive linkage (e.g., via a cysteine in the antibody). In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046).

[000463] Another example of a structure of a complex comprising an anti-TfR antibody covalently linked to a molecular payload via a Val-cit linker is provided below:



(D)

wherein n is a number between 0-10, wherein m is a number between 0-10, wherein the linker is linked to the antibody via an amine group (e.g., on a lysine residue), and/or (e.g., and) wherein the linker is linked to the oligonucleotide (e.g., at the 5' end, 3' end, or internally). In some embodiments, the linker is linked to the antibody via a lysine, the linker is linked to the oligonucleotide at the 5' end, n is 3, and m is 4. In some embodiments, the molecular payload is an oligonucleotide comprising a sense strand and an antisense strand, and, the linker is linked to the sense strand or the antisense strand at the 5' end or the 3' end.

[000464] It should be appreciated that antibodies can be linked to molecular payloads with different stochiometries, a property that may be referred to as a drug to antibody ratios (DAR) with the "drug" being the molecular payload. In some embodiments, one molecular payload is linked to an antibody (DAR = 1). In some embodiments, two molecular payloads are linked to an antibody (DAR = 2). In some embodiments, three molecular payloads are linked to an antibody (DAR = 3). In some embodiments, four molecular payloads are linked to an antibody (DAR = 4). In some embodiments, a mixture of different complexes, each having a different DAR, is provided. In some embodiments, an average DAR of complexes in such a mixture may be in a range of 1 to 3, 1 to 4, 1 to 5 or more. DAR may be increased by conjugating molecular payloads to different sites on an antibody and/or (e.g., and) by conjugating multimers to one or more sites on antibody. For example, a DAR of 2 may be

achieved by conjugating a single molecular payload to two different sites on an antibody or by conjugating a dimer molecular payload to a single site of an antibody.

[000465] In some embodiments, the complex described herein comprises an anti-TfR antibody described herein (e.g., the 3-A4, 3-M12, and 5-H12 antibodies provided in Table 2 in an IgG or Fab form) covalently linked to a molecular payload. In some embodiments, the complex described herein comprises an anti-TfR antibody described herein (e.g., the 3-A4, 3-M12, and 5-H12 antibodies provided in Table 2 in an IgG or Fab form) covalently linked to molecular payload via a linker (e.g., a Val-cit linker). In some embodiments, the linker (e.g., a Val-cit linker) is linked to the antibody (e.g., an anti-TfR antibody described herein) via a thiol-reactive linkage (e.g., via a cysteine in the antibody). In some embodiments, the linker (e.g., a Val-cit linker) is linked to the antibody (e.g., an anti-TfR antibody described herein) via an amine group (e.g., via a lysine in the antibody). In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046).

[000466] In some embodiments, in any one of the examples of complexes described herein, the molecular payload is a DMD targeting oligonucleotide comprising a region of complementarity of at least 15 consecutive nucleotides to a target sequence provided by any one of SEQ ID NO: 1242-2046. In some embodiments, in any one of the examples of complexes described herein, the molecular payload is a DMD targeting oligonucleotide comprising a region of at least 15 consecutive nucleotides of any one of SEQ ID NO: 437-1241. In some embodiments, in any one of the examples of complexes described herein, the molecular payload is a DMD targeting oligonucleotide comprising a region of complementarity of at least 5 consecutive nucleotides of an ESE listed in Table 15. In some embodiments, in any one of the examples of complexes described herein, the molecular payload is a DMD targeting oligonucleotide selected from the oligonucleotides listed in Table 14. In some embodiments, in any one of the examples of complexes described herein, the molecular payload is a DMD targeting oligonucleotide selected from the oligonucleotides provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000467] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 that are the same as the CDR-H1, CDR-H2, and CDR-

H3 shown in Table 2; and a CDR-L1, a CDR-L2, and a CDR-L3 that are the same as the CDR-L1, CDR-L2, and CDR-L3 shown in Table 2. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000468] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 69, SEQ ID NO: 71, or SEQ ID NO: 72, and a VL comprising the amino acid sequence of SEQ ID NO: 70. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000469] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 73 or SEQ ID NO: 76, and a VL comprising the amino acid sequence of SEQ ID NO: 74. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000470] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 73 or SEQ ID NO: 76, and a VL comprising the amino acid sequence of SEQ ID NO: 75. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000471] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 77, and a VL comprising the amino acid sequence of SEQ ID NO: 78. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000472] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 77 or SEQ ID NO: 79, and a VL comprising the amino acid sequence of SEQ ID NO: 80. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000473] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 84, SEQ ID NO: 86 or SEQ ID NO: 87 and a light chain comprising the amino acid sequence of SEQ ID NO: 85. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000474] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 88 or SEQ ID NO: 91, and a light chain comprising the amino acid sequence of SEQ ID NO: 89. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000475] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 88 or SEQ ID NO: 91, and a light chain comprising the amino acid sequence of SEQ ID NO: 90. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000476] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 92 or SEQ ID NO: 94, and a light chain comprising the amino acid sequence of SEQ ID NO: 95. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table

14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000477] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 92, and a light chain comprising the amino acid sequence of SEQ ID NO: 93. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000478] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload,, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 97, SEQ ID NO: 98, or SEQ ID NO: 99 and a VL comprising the amino acid sequence of SEQ ID NO: 85. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000479] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 100 or SEQ ID NO: 101 and a light chain comprising the amino acid sequence of SEQ ID NO: 89. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

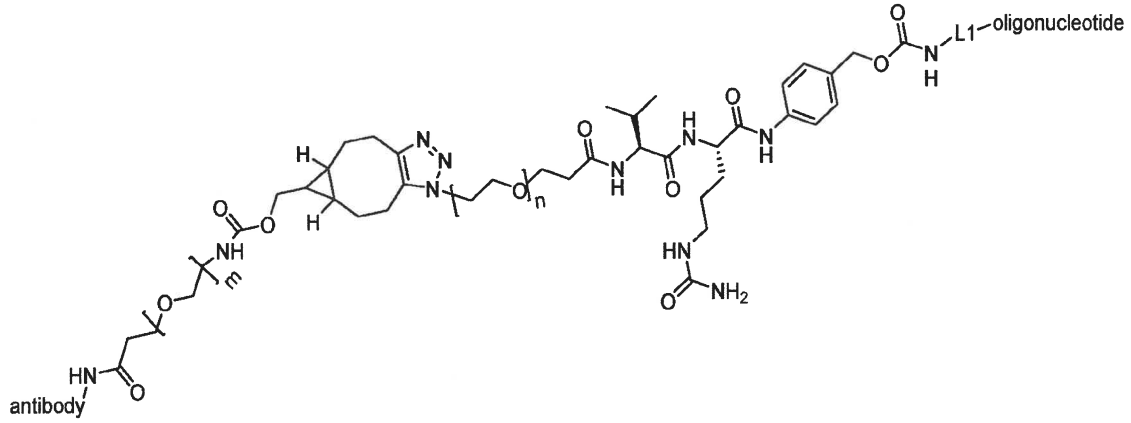
[000480] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 100 or SEQ ID NO: 101 and a light chain comprising the amino acid sequence of SEQ ID NO: 90. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000481] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 and a light chain

comprising the amino acid sequence of SEQ ID NO: 93. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000482] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 or SEQ ID NO: 103 and a light chain comprising the amino acid sequence of SEQ ID NO: 95. In some embodiments, the molecular payload is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the molecular payload is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

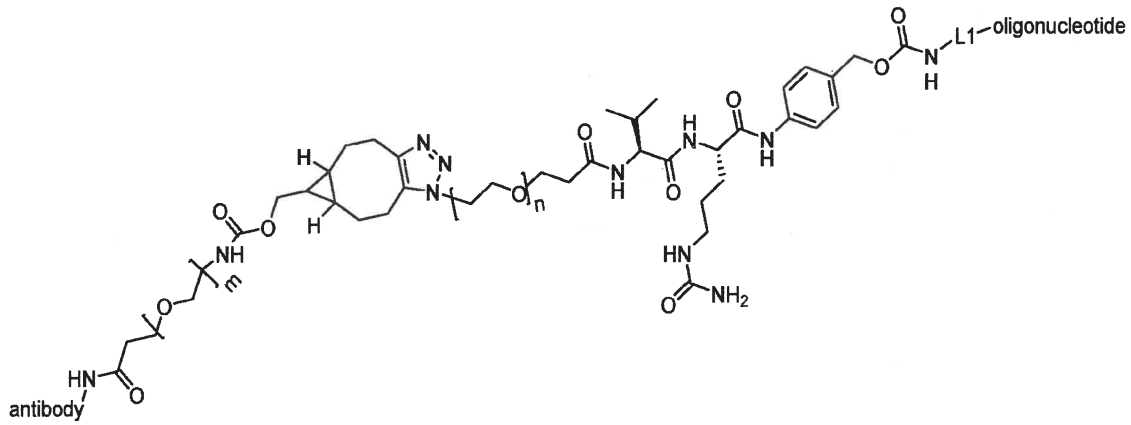
[000483] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 84 and a light chain comprising the amino acid sequence of in SEQ ID NO: 85; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

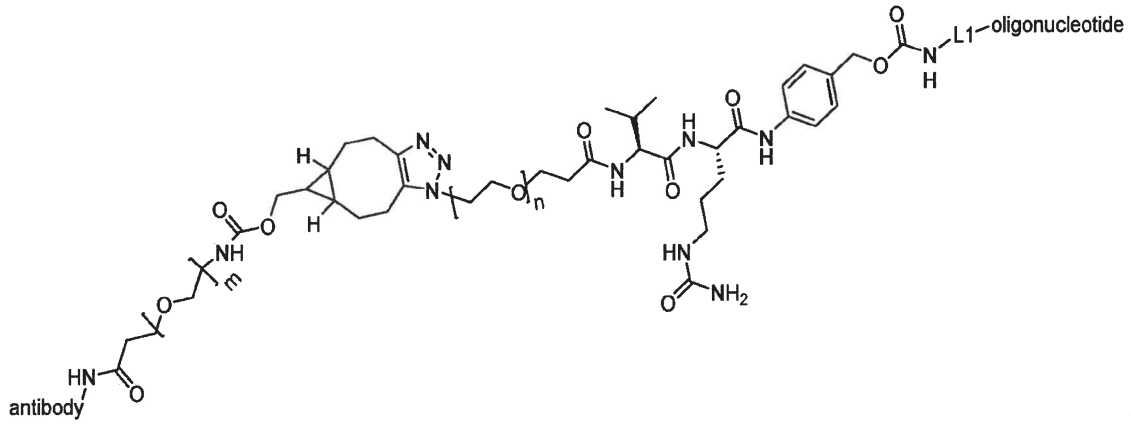
[000484] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 86 and a light chain comprising the amino acid sequence of in SEQ ID NO: 85; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000485] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 87 and a light chain comprising the amino acid sequence of in SEQ ID NO: 85; wherein the complex has the structure of:

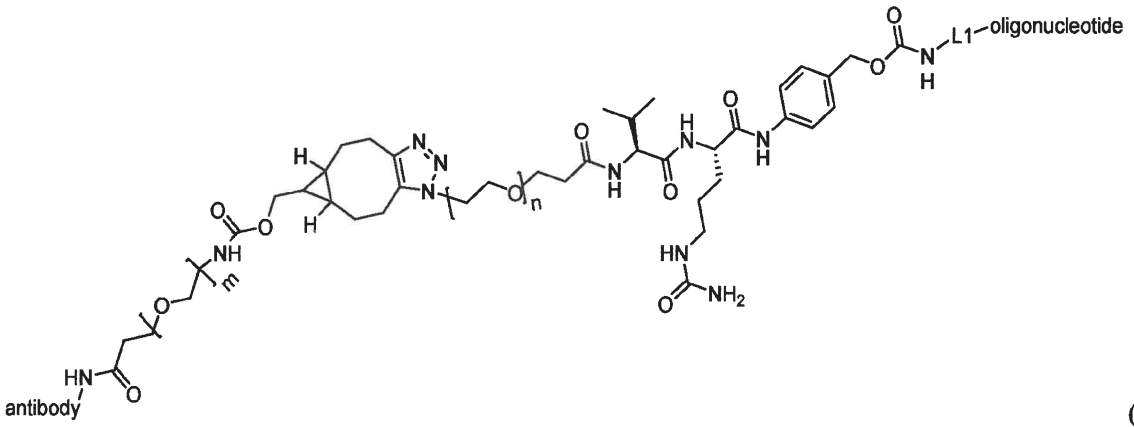


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000486] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 88

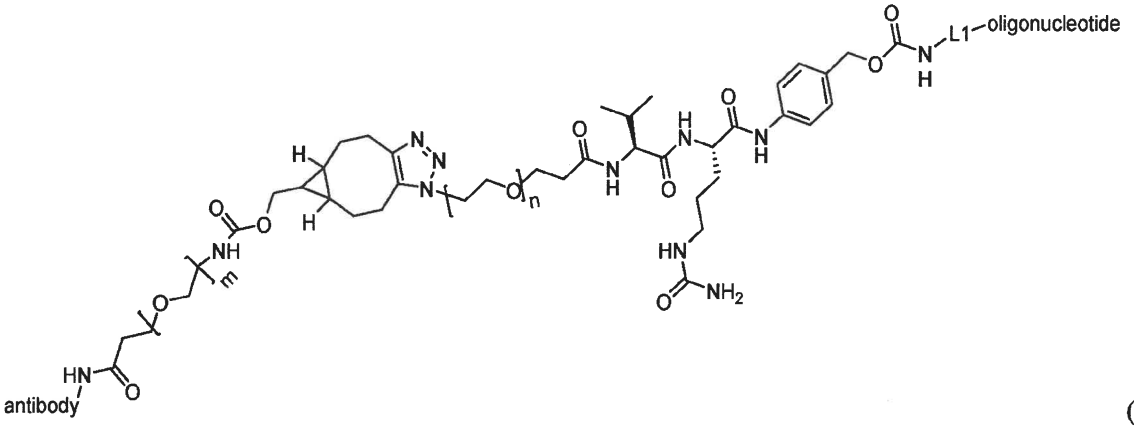
and a light chain comprising the amino acid sequence of in SEQ ID NO: 89; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000487] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 88 and a light chain comprising the amino acid sequence of in SEQ ID NO: 90; wherein the complex has the structure of:

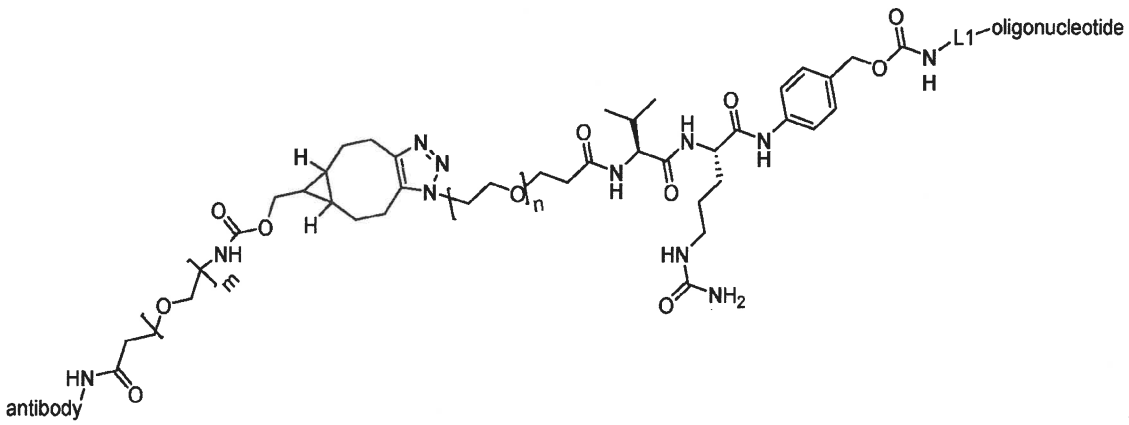


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000488] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-

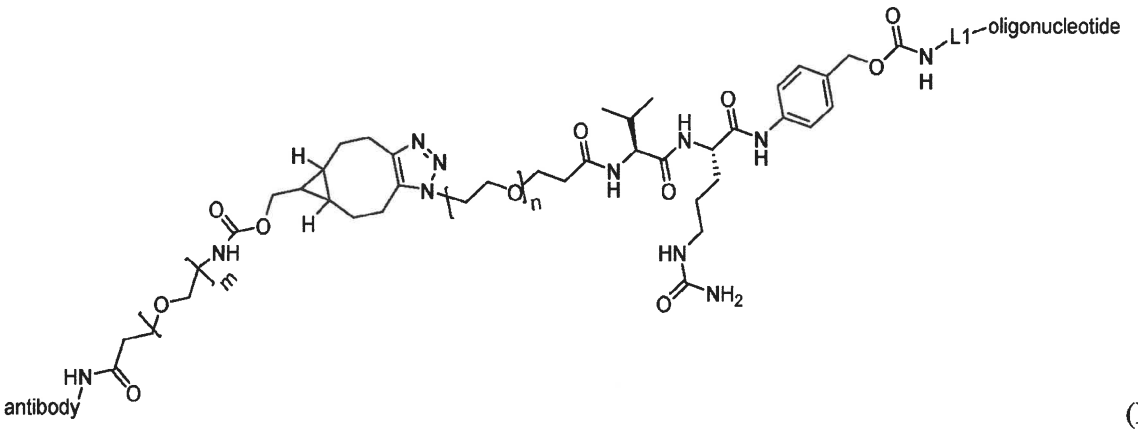
TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of in SEQ ID NO: 89; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

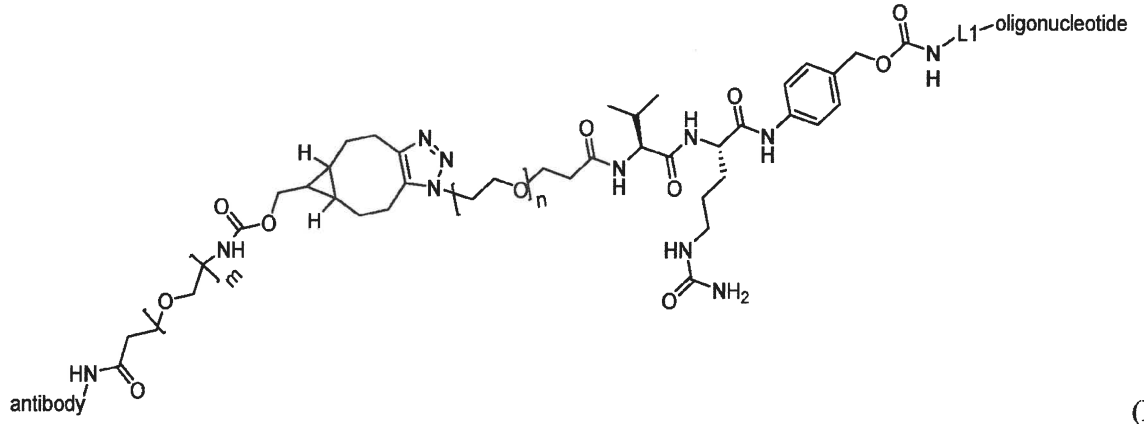
[000489] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 91 and a light chain comprising the amino acid sequence of in SEQ ID NO: 90; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

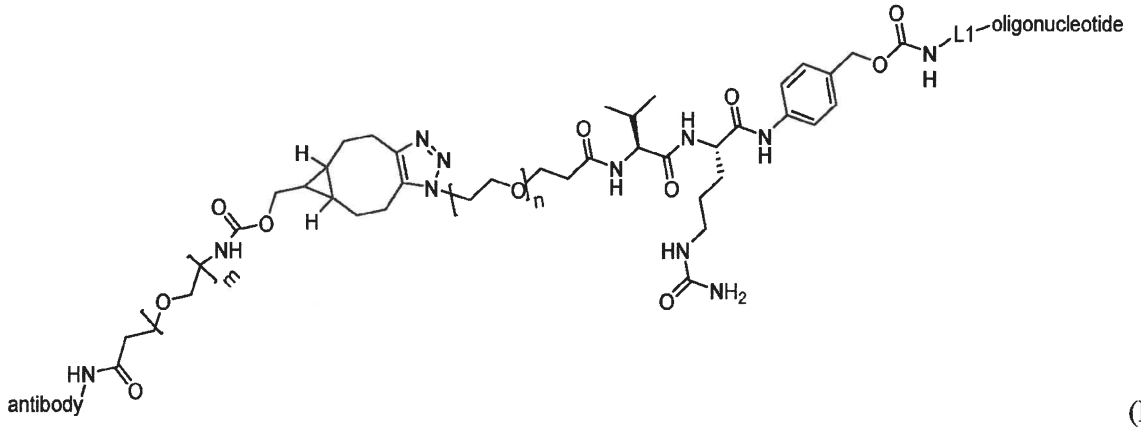
[000490] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 92 and a light chain comprising the amino acid sequence of in SEQ ID NO: 93; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000491] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 94 and a light chain comprising the amino acid sequence of in SEQ ID NO: 95; wherein the complex has the structure of:

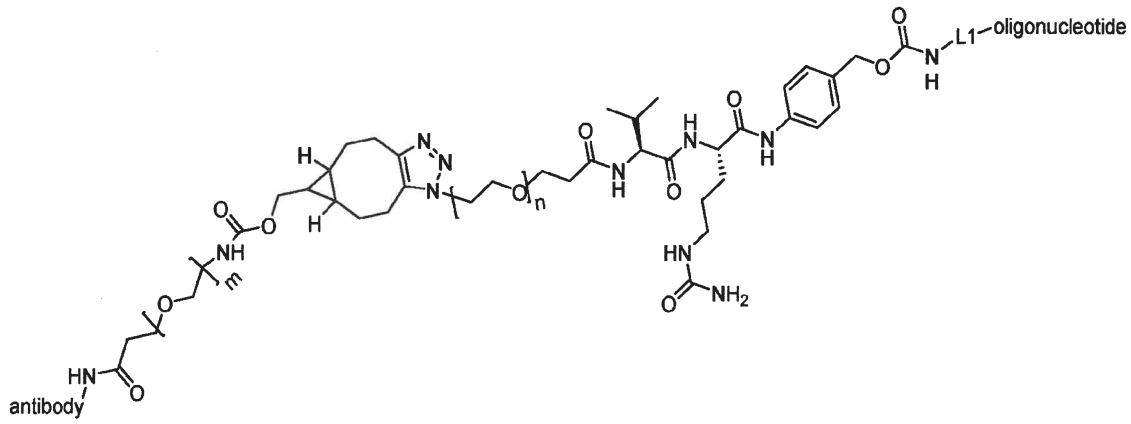


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the

oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

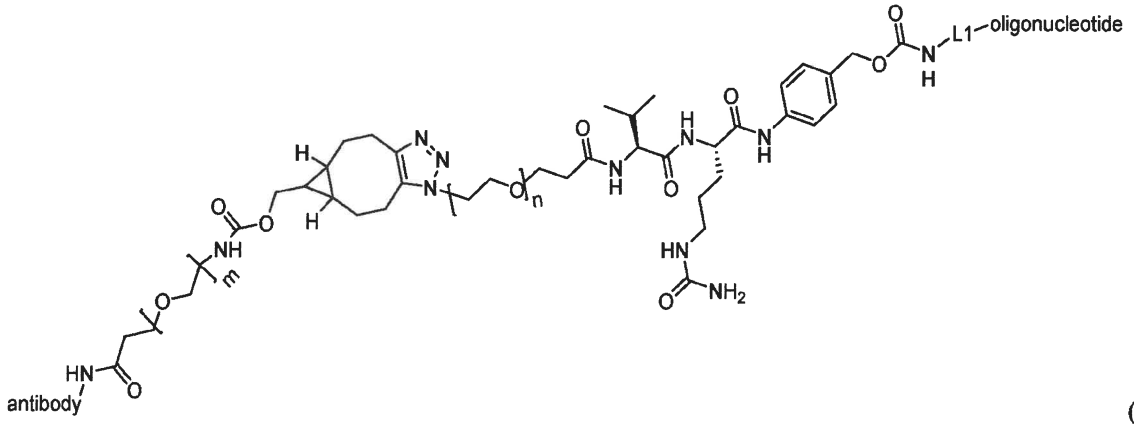
[000492] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 92 and a light chain comprising the amino acid sequence of in SEQ ID NO: 95; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000493] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 69 and a VL comprising the amino acid sequence of in SEQ ID NO: 70; wherein the complex has the structure of:

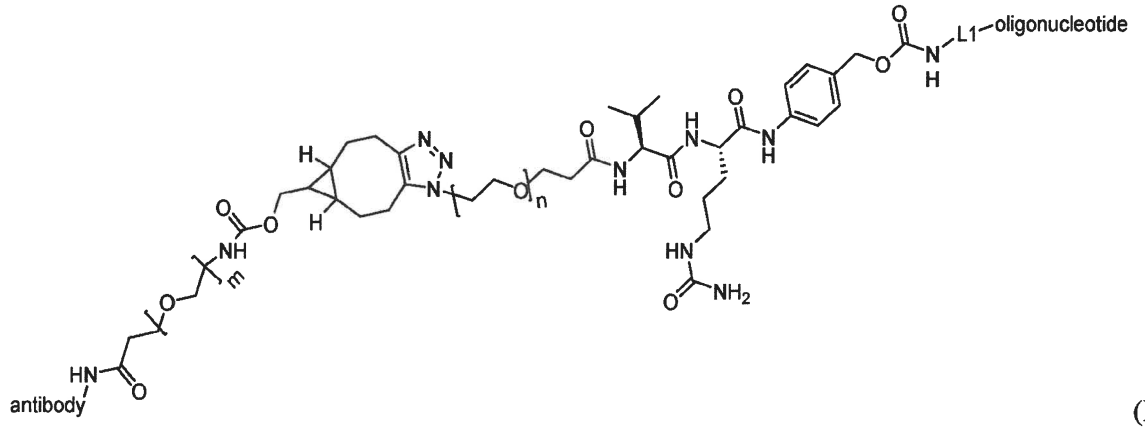


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the

oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

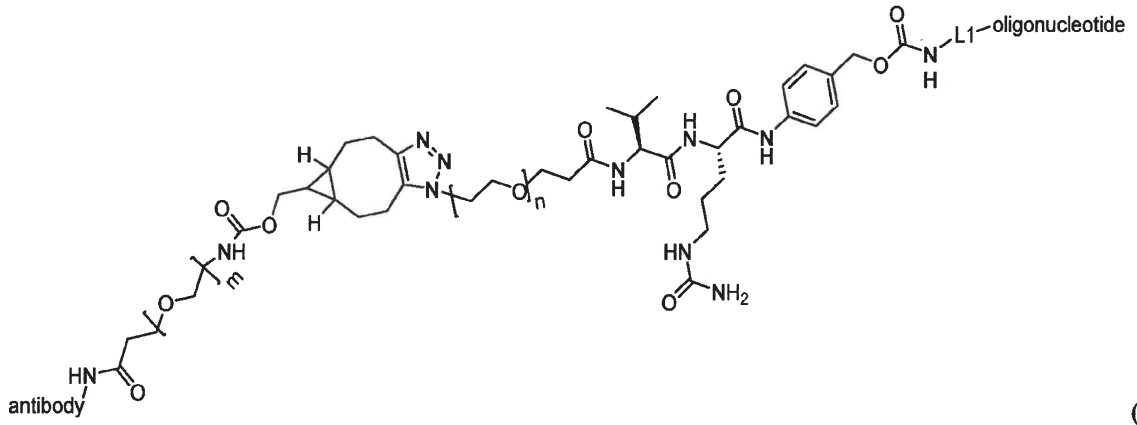
[000494] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 71 and a VL comprising the amino acid sequence of in SEQ ID NO: 70; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000495] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 72 and a VL comprising the amino acid sequence of in SEQ ID NO: 70; wherein the complex has the structure of:

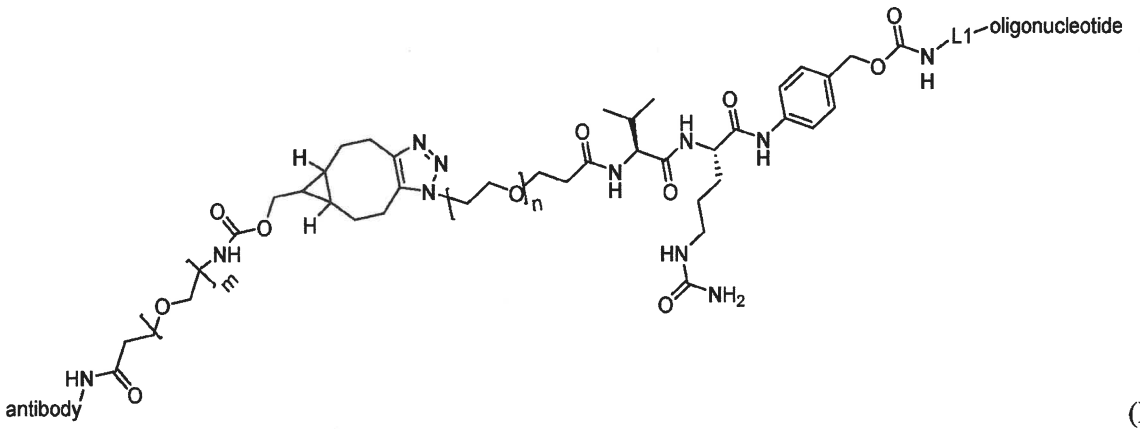


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the

oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

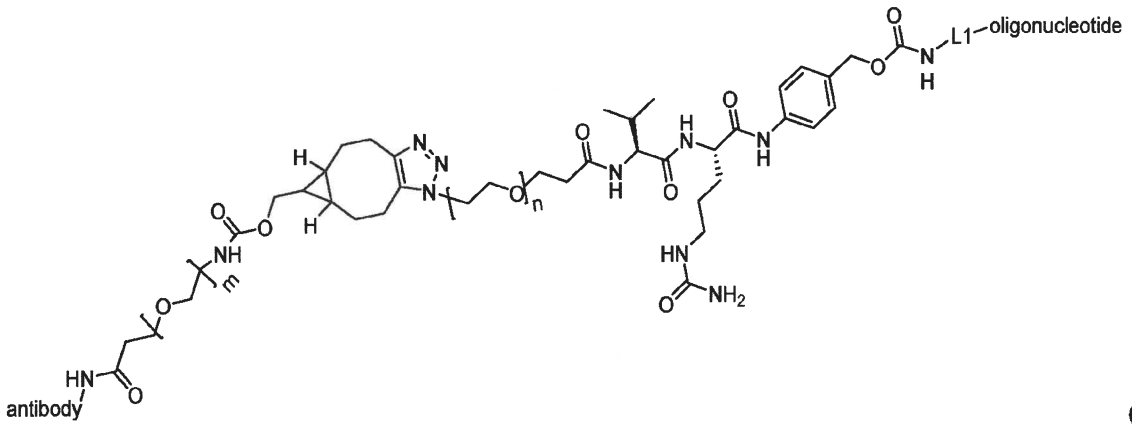
[000496] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of in SEQ ID NO: 74; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000497] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of in SEQ ID NO: 75; wherein the complex has the structure of:

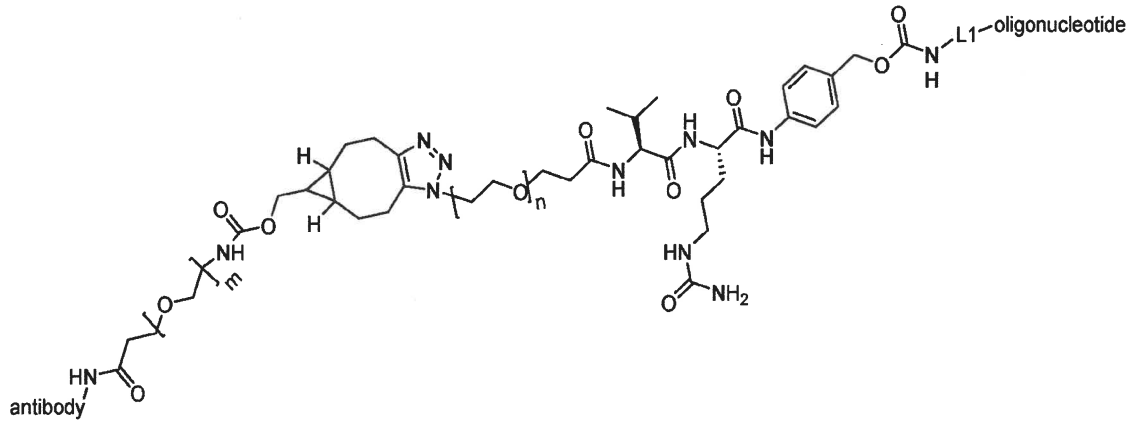


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the

oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

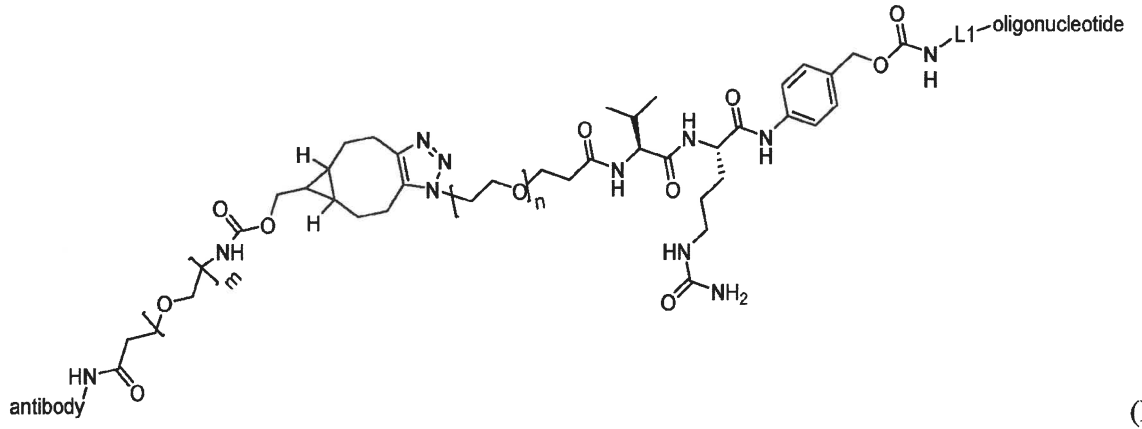
[000498] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 76 and a VL comprising the amino acid sequence of in SEQ ID NO: 74; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000499] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 76 and a VL comprising the amino acid sequence of in SEQ ID NO: 75; wherein the complex has the structure of:

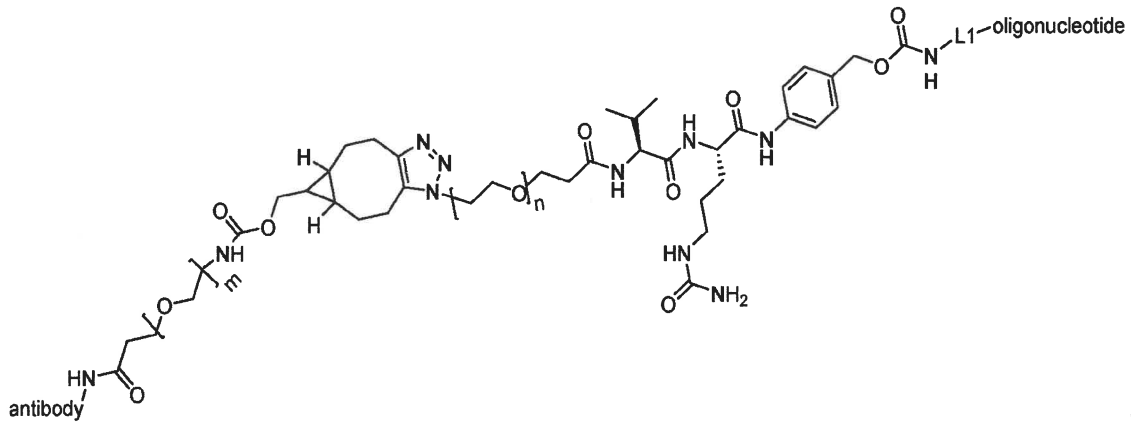


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the

oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

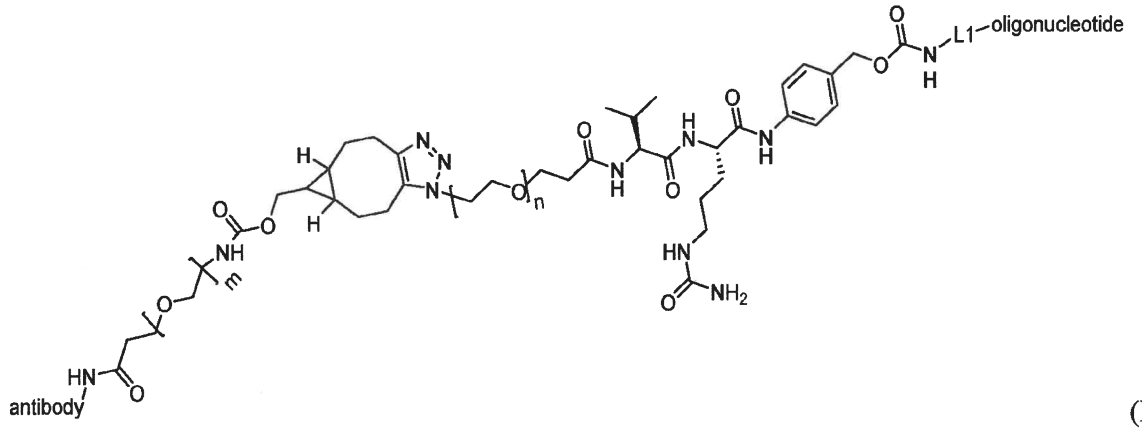
[000500] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 77 and a VL comprising the amino acid sequence of in SEQ ID NO: 78; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000501] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 79 and a VL comprising the amino acid sequence of in SEQ ID NO: 80; wherein the complex has the structure of:

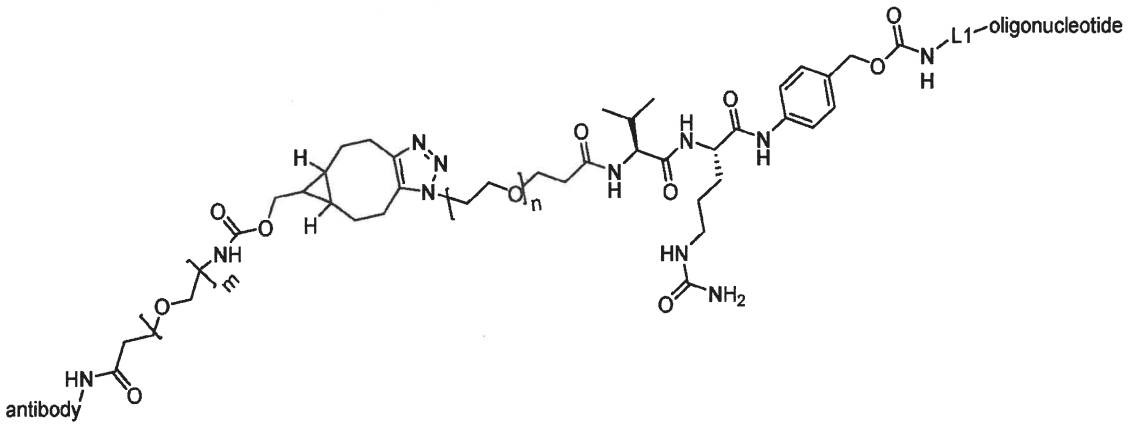


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the

oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

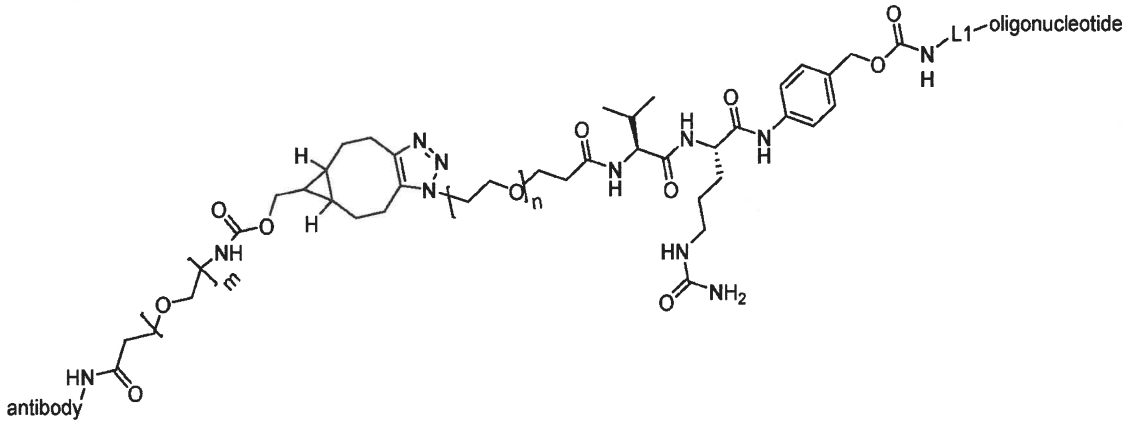
[000502] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a VH comprising the amino acid sequence of SEQ ID NO: 77 and a VL comprising the amino acid sequence of in SEQ ID NO: 80; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000503] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 97 and a light chain comprising the amino acid sequence of in SEQ ID NO: 85; wherein the complex has the structure of:

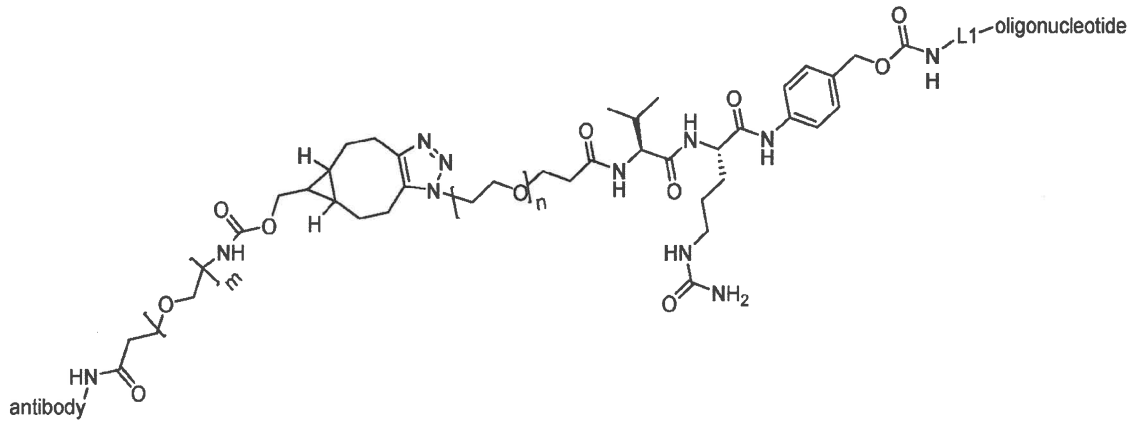


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the

oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

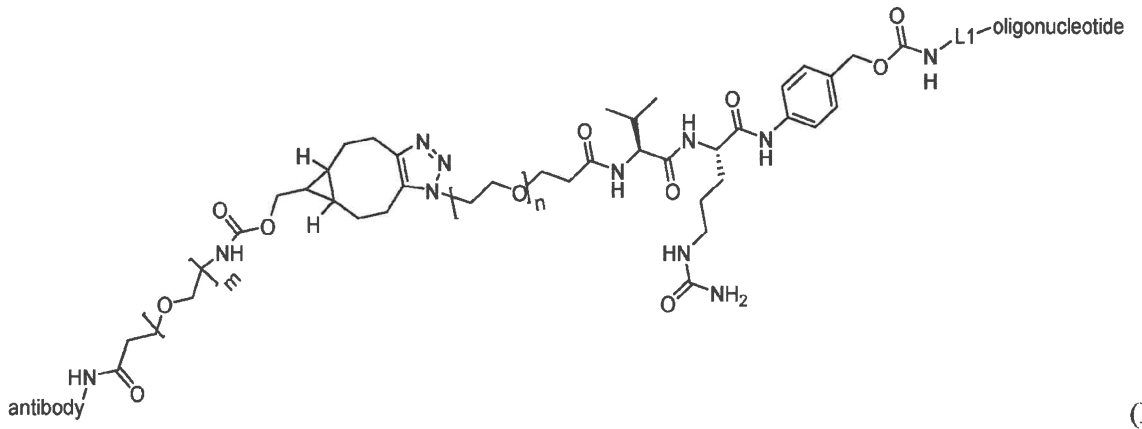
[000504] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 98 and a light chain comprising the amino acid sequence of in SEQ ID NO: 85; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000505] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 99 and a light chain comprising the amino acid sequence of in SEQ ID NO: 85; wherein the complex has the structure of:

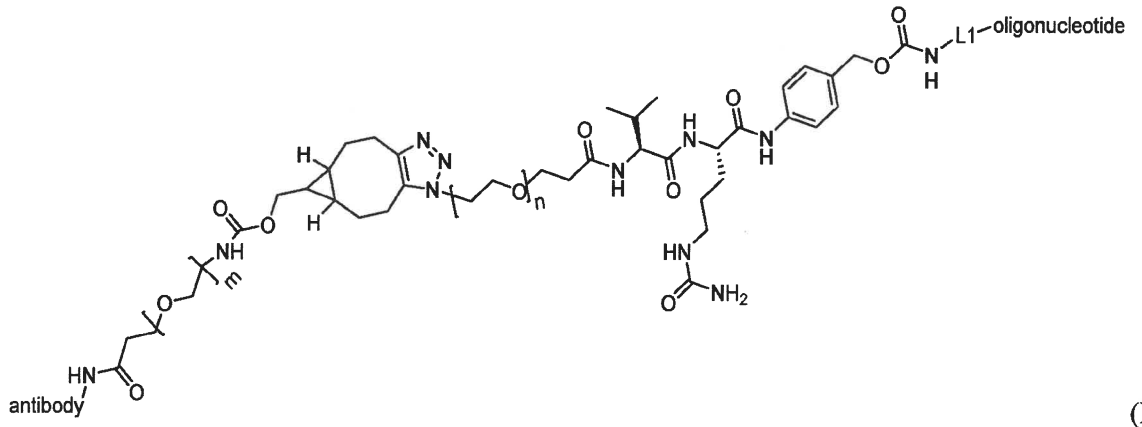


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting

oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

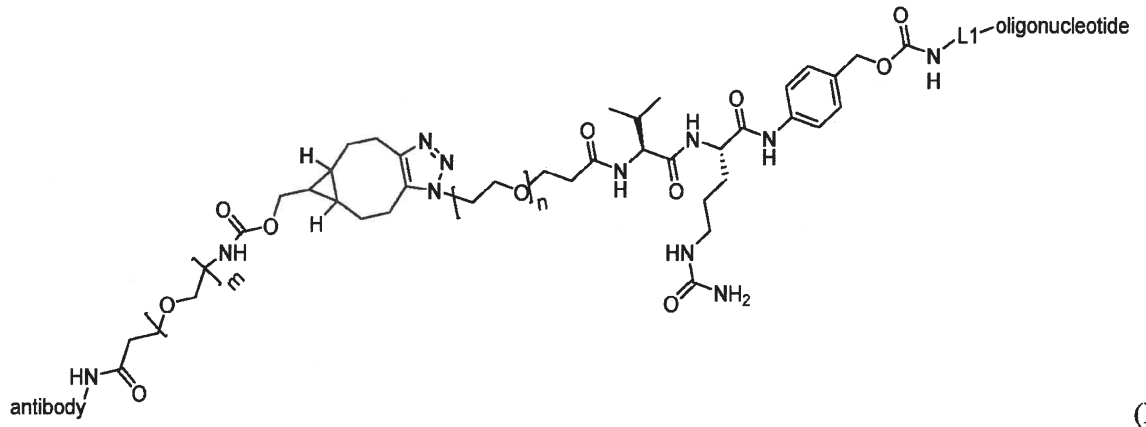
[000506] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 100 and a light chain comprising the amino acid sequence of in SEQ ID NO: 89; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

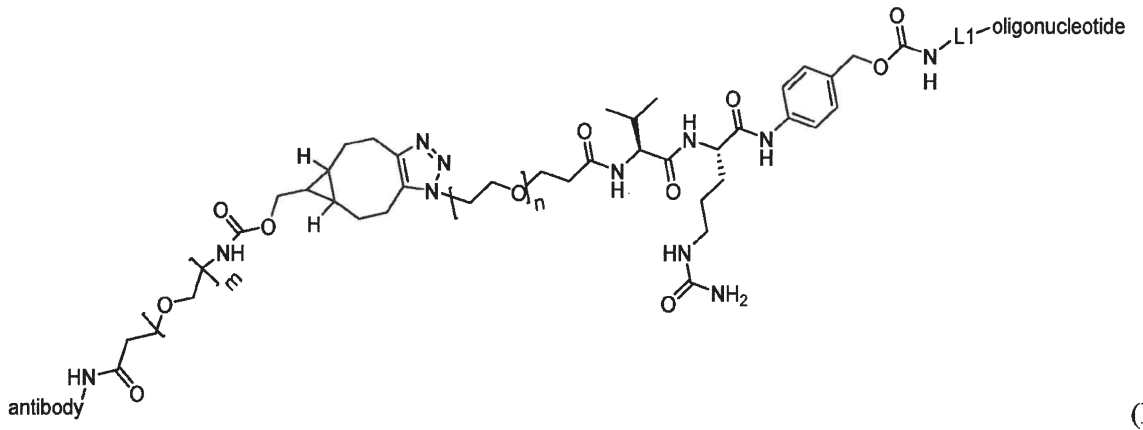
[000507] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 100 and a light chain comprising the amino acid sequence of in SEQ ID NO: 90; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000508] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 101 and a light chain comprising the amino acid sequence of in SEQ ID NO: 89; wherein the complex has the structure of:

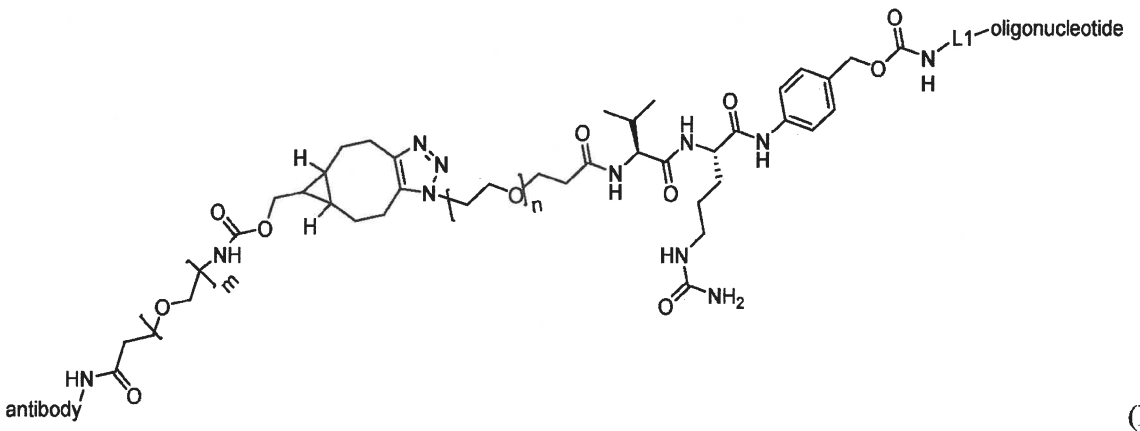


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000509] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 101 and a light

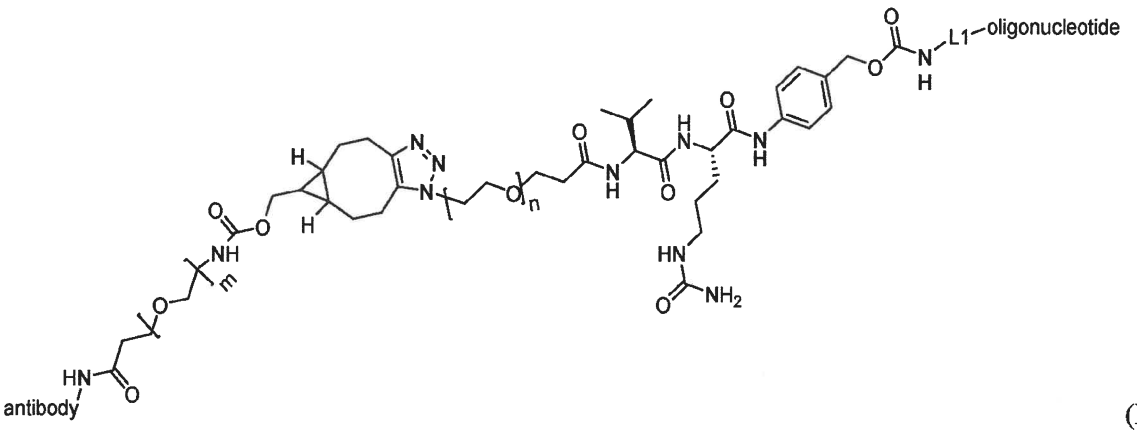
chain comprising the amino acid sequence of in SEQ ID NO: 90; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000510] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 and a light chain comprising the amino acid sequence of in SEQ ID NO: 93; wherein the complex has the structure of:

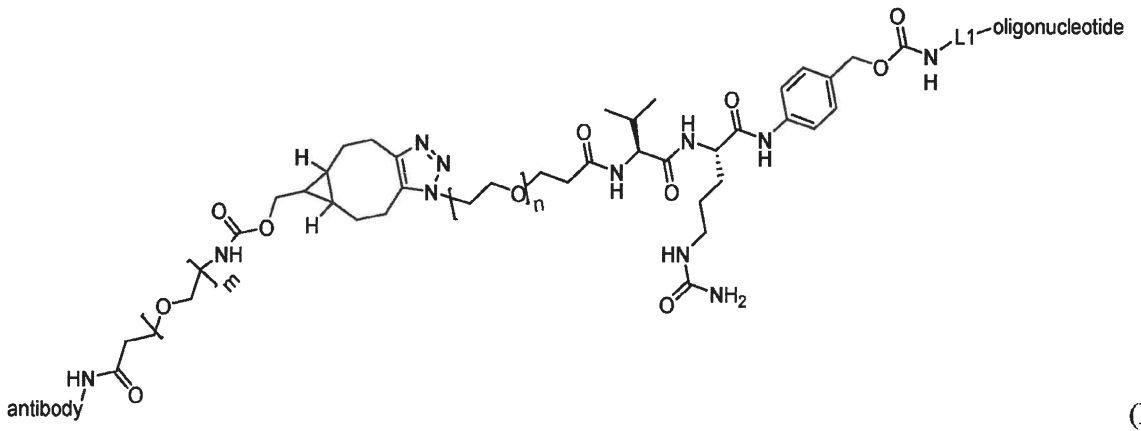


(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000511] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab

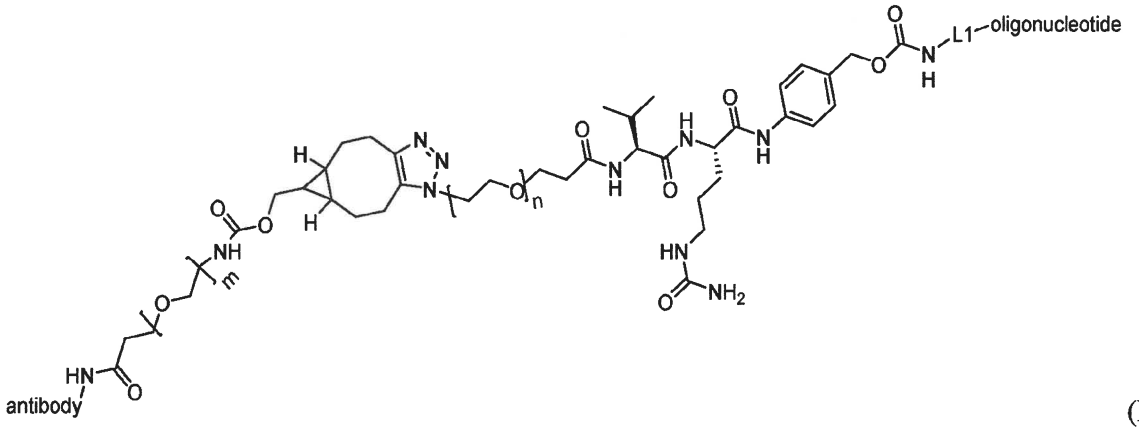
comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 103 and a light chain comprising the amino acid sequence of in SEQ ID NO: 95; wherein the complex has the structure of:



(D)

wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000512] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 and a light chain comprising the amino acid sequence of in SEQ ID NO: 95; wherein the complex has the structure of:

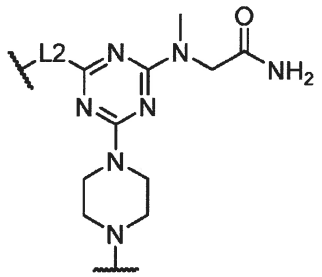


(D)

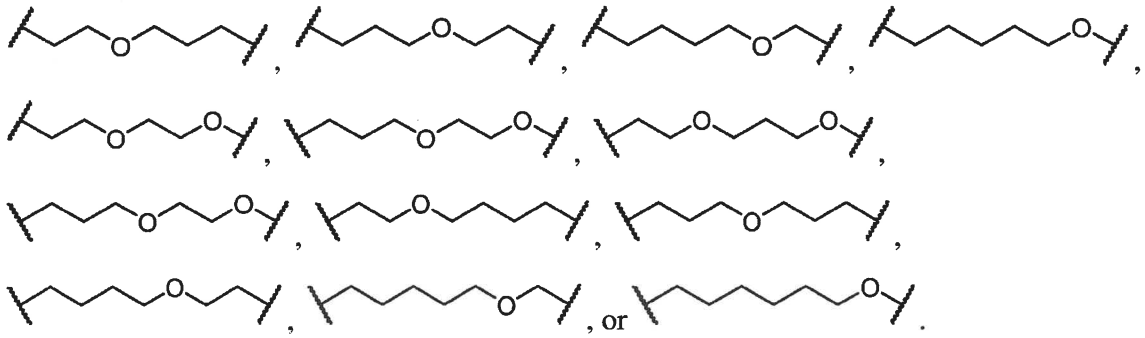
wherein n is 3 and m is 4. In some embodiments, the oligonucleotide is a DMD targeting oligonucleotide (e.g., an oligonucleotide listed in Table 14). In some embodiments, the oligonucleotide is an oligonucleotide provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

[000513] In some embodiments, in any one of the examples of complexes described herein, L1 is any one of the spacers described herein.

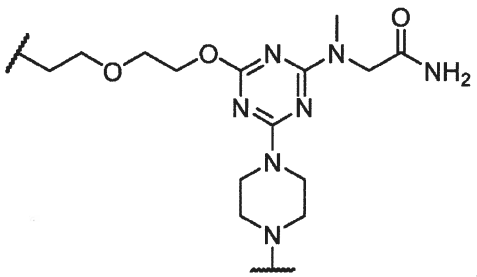
[000514] In some embodiments, L1 is:



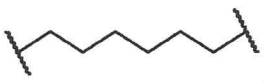
wherein the piperazine moiety links to the oligonucleotide, wherein L2 is



[000515] In some embodiments, L1 is:



wherein the piperazine moiety links to the oligonucleotide.

[000516] In some embodiments, L1 is .

[000517] In some embodiments, L1 is linked to a 5' phosphate of the oligonucleotide.

[000518] In some embodiments, L1 is optional (e.g., need not be present).

III. Formulations

[000519] Complexes provided herein may be formulated in any suitable manner. Generally, complexes provided herein are formulated in a manner suitable for pharmaceutical use. For example, complexes can be delivered to a subject using a formulation that minimizes degradation, facilitates delivery and/or (e.g., and) uptake, or provides another beneficial property to the complexes in the formulation. In some embodiments, provided herein are

compositions comprising complexes and pharmaceutically acceptable carriers. Such compositions can be suitably formulated such that when administered to a subject, either into the immediate environment of a target cell or systemically, a sufficient amount of the complexes enter target muscle cells. In some embodiments, complexes are formulated in buffer solutions such as phosphate-buffered saline solutions, liposomes, micellar structures, and capsids.

[000520] It should be appreciated that, in some embodiments, compositions may include separately one or more components of complexes provided herein (e.g., muscle-targeting agents, linkers, molecular payloads, or precursor molecules of any one of them).

[000521] In some embodiments, complexes are formulated in water or in an aqueous solution (e.g., water with pH adjustments). In some embodiments, complexes are formulated in basic buffered aqueous solutions (e.g., PBS). In some embodiments, formulations as disclosed herein comprise an excipient. In some embodiments, an excipient confers to a composition improved stability, improved absorption, improved solubility and/or (e.g., and) therapeutic enhancement of the active ingredient. In some embodiments, an excipient is a buffering agent (*e.g.*, sodium citrate, sodium phosphate, a tris base, or sodium hydroxide) or a vehicle (*e.g.*, a buffered solution, petrolatum, dimethyl sulfoxide, or mineral oil).

[000522] In some embodiments, a complex or component thereof (e.g., oligonucleotide or antibody) is lyophilized for extending its shelf-life and then made into a solution before use (*e.g.*, administration to a subject). Accordingly, an excipient in a composition comprising a complex, or component thereof, described herein may be a lyoprotectant (*e.g.*, mannitol, lactose, polyethylene glycol, or polyvinyl pyrolidone), or a collapse temperature modifier (*e.g.*, dextran, ficoll, or gelatin).

[000523] In some embodiments, a pharmaceutical composition is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, administration. Typically, the route of administration is intravenous or subcutaneous.

[000524] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. In some embodiments, formulations include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, and sodium chloride in the composition. Sterile injectable solutions

can be prepared by incorporating the complexes in a required amount in a selected solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

[000525] In some embodiments, a composition may contain at least about 0.1% of the a complex, or component thereof, or more, although the percentage of the active ingredient(s) may be between about 1% and about 80% or more of the weight or volume of the total composition. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

IV. Methods of Use / Treatment

[000526] Complexes comprising a muscle-targeting agent covalently linked to a molecular payload as described herein are effective in treating a subject having a dystrophinopathy, e.g., Duchenne muscular dystrophy. In some embodiments, complexes comprise a molecular payload that is an oligonucleotide, *e.g.*, an antisense oligonucleotide that facilitates exon skipping of an mRNA expressed from a mutated DMD allele.

[000527] In some embodiments, a subject may be a human subject, a non-human primate subject, a rodent subject, or any suitable mammalian subject. In some embodiments, a subject may have Duchenne muscular dystrophy or other dystrophinopathy. In some embodiments, a subject has a mutated DMD allele, which may optionally comprise at least one mutation in a DMD exon that causes a frameshift mutation and leads to improper RNA splicing/processing. In some embodiments, a subject is suffering from symptoms of a severe dystrophinopathy, e.g. muscle atrophy or muscle loss. In some embodiments, a subject has an asymptomatic increase in serum concentration of creatine phosphokinase (CK) and/or (e.g., and) muscle cramps with myoglobinuria. In some embodiments, a subject has a progressive muscle disease, such as Duchenne or Becker muscular dystrophy or DMD-associated dilated cardiomyopathy (DCM). In some embodiments, a subject is not suffering from symptoms of a dystrophinopathy.

[000528] An aspect of the disclosure includes a methods involving administering to a subject an effective amount of a complex as described herein. In some embodiments, an effective amount of a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently linked to a molecular payload can be administered to a subject in need of treatment. In some embodiments, a pharmaceutical composition comprising a complex as described herein may be administered by a suitable route, which may include

intravenous administration, e.g., as a bolus or by continuous infusion over a period of time. In some embodiments, intravenous administration may be performed by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, or intrathecal routes. In some embodiments, a pharmaceutical composition may be in solid form, aqueous form, or a liquid form. In some embodiments, an aqueous or liquid form may be nebulized or lyophilized. In some embodiments, a nebulized or lyophilized form may be reconstituted with an aqueous or liquid solution.

[000529] Compositions for intravenous administration may contain various carriers such as vegetable oils, dimethylactamide, dimethyformamide, ethyl lactate, ethyl carbonate, isopropyl myristate, ethanol, and polyols (glycerol, propylene glycol, liquid polyethylene glycol, and the like). For intravenous injection, water soluble antibodies can be administered by the drip method, whereby a pharmaceutical formulation containing the antibody and a physiologically acceptable excipients is infused. Physiologically acceptable excipients may include, for example, 5% dextrose, 0.9% saline, Ringer's solution or other suitable excipients. Intramuscular preparations, e.g., a sterile formulation of a suitable soluble salt form of the antibody, can be dissolved and administered in a pharmaceutical excipient such as Water-for-Injection, 0.9% saline, or 5% glucose solution.

[000530] In some embodiments, a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently linked to a molecular payload is administered via site-specific or local delivery techniques. Examples of these techniques include implantable depot sources of the complex, local delivery catheters, site specific carriers, direct injection, or direct application.

[000531] In some embodiments, a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently linked to a molecular payload is administered at an effective concentration that confers therapeutic effect on a subject. Effective amounts vary, as recognized by those skilled in the art, depending on the severity of the disease, unique characteristics of the subject being treated, e.g. age, physical conditions, health, or weight, the duration of the treatment, the nature of any concurrent therapies, the route of administration and related factors. These related factors are known to those in the art and may be addressed with no more than routine experimentation. In some embodiments, an effective concentration is the maximum dose that is considered to be safe for the patient. In some embodiments, an effective concentration will be the lowest possible concentration that provides maximum efficacy.

[000532] Empirical considerations, e.g. the half-life of the complex in a subject, generally will contribute to determination of the concentration of pharmaceutical composition that is used for treatment. The frequency of administration may be empirically determined and adjusted to maximize the efficacy of the treatment.

[000533] Generally, for administration of any of the complexes described herein, an initial candidate dosage may be about 1 to 100 mg/kg, or more, depending on the factors described above, e.g. safety or efficacy. In some embodiments, a treatment will be administered once. In some embodiments, a treatment will be administered daily, biweekly, weekly, bimonthly, monthly, or at any time interval that provide maximum efficacy while minimizing safety risks to the subject. Generally, the efficacy and the treatment and safety risks may be monitored throughout the course of treatment

[000534] The efficacy of treatment may be assessed using any suitable methods. In some embodiments, the efficacy of treatment may be assessed by evaluation of observation of symptoms associated with a dystrophinopathy, e.g. muscle atrophy or muscle weakness, through measures of a subject's self-reported outcomes, e.g. mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, or by quality-of-life indicators, e.g. lifespan.

[000535] In some embodiments, a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently linked to a molecular payload described herein is administered to a subject at an effective concentration sufficient to modulate activity or expression of a target gene by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90% or at least 95% relative to a control, e.g. baseline level of gene expression prior to treatment.

[000536] In some embodiments, a single dose or administration of a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently linked to a molecular payload described herein to a subject is sufficient to inhibit activity or expression of a target gene for at least 1-5, 1-10, 5-15, 10-20, 15-30, 20-40, 25-50, or more days. In some embodiments, a single dose or administration of a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently linked to a molecular payload described herein to a subject is sufficient to inhibit activity or expression of a target gene for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks. In some embodiments, a single dose or administration of a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently linked to a molecular payload described herein to a subject

is sufficient to inhibit activity or expression of a target gene for at least 1, 2, 3, 4, 5, or 6 months.

[000537] In some embodiments, a pharmaceutical composition may comprise more than one complex comprising a muscle-targeting agent covalently linked to a molecular payload. In some embodiments, a pharmaceutical composition may further comprise any other suitable therapeutic agent for treatment of a subject, e.g. a human subject having a dystrophinopathy. In some embodiments, the other therapeutic agents may enhance or supplement the effectiveness of the complexes described herein. In some embodiments, the other therapeutic agents may function to treat a different symptom or disease than the complexes described herein.

EXAMPLES

Example 1: Targeting HPRT with transfected antisense oligonucleotides

[000538] A siRNA that targets hypoxanthine phosphoribosyltransferase (HPRT) was tested *in vitro* for its ability to reduce expression levels of HPRT in an immortalized cell line. Briefly, Hepa 1-6 cells were transfected with either a control siRNA (siCTRL; 100 nM) or the siRNA that targets HPRT (siHPRT; 100 nM), formulated with lipofectamine 2000. HPRT expression levels were evaluated 48 hours following transfection. A control experiment was also performed in which vehicle (phosphate-buffered saline) was delivered to Hepa 1-6 cells in culture and the cells were maintained for 48 hours. As shown in FIG. 1, it was found that the HPRT siRNA reduced HPRT expression levels by about 90% compared with controls. Sequences of the siRNAs used are provided in Table 6.

Table 6. Sequences of siHPRT and siCTRL

	Sequence*	SEQ ID NO:
siHPRT sense strand	5'-UcCuAuGaCuGuAgAuUuUaU-$(CH_2)_6NH_2$-3′	147
siHPRT antisense strand	5'-paUaAaAuCuAcAgUcAuAgGasAsu-3′	148
siCTRL sense strand	5'-UgUaAuAaCcAuAuCuAcCuU-$(CH_2)_6NH_2$-3′	149
siCTRL antisense strand	5'-aAgGuAgAuAuGgUuAuUaCasAsa-3′	150

*Lower case - 2'Ome ribose; Capital letter - 2'Fluoro ribose; p – phosphate linkage; s – phosphorothioate linkage

Example 2: Targeting HPRT with a muscle-targeting complex

[000539] A muscle-targeting complex was generated comprising the HPRT siRNA used in Example 1 (siHPRT) covalently linked, via a non-cleavable N-gamma-maleimidobutyryl-

oxysuccinimide ester (GMBS) linker, to RI7 217 anti-TfR1 Fab (DTX-A-002), an anti-transferrin receptor antibody.

[000540] Briefly, the GMBS linker was dissolved in dry DMSO and coupled to the 3' end of the sense strand of siHPRT through amide bond formation under aqueous conditions. Completion of the reaction was verified by Kaiser test. Excess linker and organic solvents were removed by gel permeation chromatography. The purified, maleimide functionalized sense strand of siHPRT was then coupled to DTX-A-002 antibody using a Michael addition reaction.

[000541] The product of the antibody coupling reaction was then subjected to size exclusion chromatograpy (SEC) purification. antiTfR-siHPRT complexes comprising one or two siHPRT molecules covalently attached to DTX-A-002 antibody were purified. Densitometry confirmed that the purified sample of complexes had an average siHPRT to antibody ratio of 1.46. SDS-PAGE analysis demonstrated that >90% of the purified sample of complexes comprised DTX-A-002 linked to either one or two siHPRT molecules.

[000542] Using the same methods as described above, a control IgG2a-siHPRT complex was generated comprising the HPRT siRNA used in Example 1 (siHPRT) covalently linked via the GMBS linker to an IgG2a (Fab) antibody (DTX-A-003). Densitometry confirmed that DTX-C-001 (the IgG2a-siHPRT complex) had an average siHPRT to antibody ratio of 1.46 and SDS-PAGE demonstrated that >90% of the purified sample of control complexes comprised DTX-A-003 linked to either one or two siHPRT molecules.

[000543] The antiTfR-siHPRT complex was then tested for cellular internalization and inhibition of HPRT *in cellulo*. Hepa 1-6 cells, which have relatively high expression levels of transferrin receptor, were incubated in the presence of vehicle (phosphate-buffered saline), IgG2a-siHPRT (100 nM), antiTfR-siCTRL (100 nM), or antiTfR-siHPRT (100 nM), for 72 hours. After the 72 hour incubation, the cells were isolated and assayed for expression levels of HPRT (FIG. 2). Cells treated with the antiTfR-siHPRT demonstrated a reduction in HPRT expression by about 50% relative to the cells treated with the vehicle control and to those treated with the IgG2a-siHPRT complex. Meanwhile, cells treated with either of the IgG2a-siHPRT or antiTfR-siCTRL had HPRT expression levels comparable to the vehicle control (no reduction in HPRT expression). These data indicate that the anti-transferrin receptor antibody of the antiTfR-siHPRT enabled cellular internalization of the complex, thereby allowing the siHPRT to inhibit expression of HPRT.

Example 3: Targeting HPRT in mouse muscle tissues with a muscle-targeting complex

[000544] The muscle-targeting complex described in Example 2, antiTfR-siHPRT, was tested for inhibition of HPRT in mouse tissues. C57BL/6 wild-type mice were intravenously injected with a single dose of a vehicle control (phosphate-buffered saline); siHPRT (2 mg/kg of siRNA); IgG2a-siHPRT (2 mg/kg of siRNA, corresponding to 9 mg/kg antibody complex); or antiTfR-siHPRT (2 mg/kg of siRNA, corresponding to 9 mg/kg antibody complex. Each experimental condition was replicated in four individual C57BL/6 wild-type mice. Following a three-day period after injection, the mice were euthanized and segmented into isolated tissue types. Individual tissue samples were subsequently assayed for expression levels of HPRT (FIGs. 3A-3B and 4A-4E).

[000545] Mice treated with the antiTfR-siHPRT complex demonstrated a reduction in HPRT expression in gastrocnemius (31% reduction; $p<0.05$) and heart (30% reduction; $p<0.05$), relative to the mice treated with the siHPRT control (FIGs. 3A-3B). Meanwhile, mice treated with the IgG2a-siHPRT complex had HPRT expression levels comparable to the siHPRT control (little or no reduction in HPRT expression) for all assayed muscle tissue types.

[000546] Mice treated with the antiTfR-siHPRT complex demonstrated no change in HPRT expression in non-muscle tissues such as brain, liver, lung, kidney, and spleen tissues (FIGs. 4A-4E). These data indicate that the anti-transferrin receptor antibody of the antiTfR-siHPRT complex enabled cellular internalization of the complex into muscle-specific tissues in an *in vivo* mouse model, thereby allowing the siHPRT to inhibit expression of HPRT. These data further demonstrate that the antiTfR-oligonucleotide complexes of the current disclosure are capable of specifically targeting muscle tissues.

Example 4: Targeting DMD with a muscle-targeting complex

[000547] A muscle-targeting complex is generated comprising an antisense oligonucleotide that targets a mutant allele of DMD (DMD ASO), for exon skipping, e.g., an oligonucleotide having a sequence as disclosed in Table 14, covalently linked, via a cathepsin cleavable linker, to DTX-A-002 (RI7 217 (Fab)), an anti-transferrin receptor antibody.

[000548] Briefly, purified Val-Cit-linker-DMD ASO is coupled to a functionalized antibody fragment (e.g, RI7 217 (Fab) or 15G11 (Fab)) generated through modifying ε-amine on lysine of the antibody.

[000549] The product of the antibody coupling reaction is then subjected to hydrophobic interaction chromatography (HIC-HPLC) to purify the muscle-targeting complex. Densitometry and SDS-PAGE analysis of the purified complex allow for determination of the average ratio of ASO-to-antibody and total purity, respectively.

[000550] Using the same methods as described above, a control complex is generated comprising DMD ASO covalently linked via a Val-Cit linker to an IgG2a (Fab) antibody. The purified muscle-targeting complex comprising DTX-A-002 covalently linked to DMD ASO is then tested for cellular internalization and modulation of DMD exon skipping. Disease-relevant muscle cells that have relatively high expression levels of transferrin receptor, are incubated in the presence of vehicle control (saline), muscle-targeting complex (100 nM), or control complex (100 nM) for 72 hours. After the 72 hour incubation, the cells are isolated and assayed for expression levels of DMD.

Example 5: Targeting DMD with a muscle-targeting complex

[000551] A muscle-targeting complex (DTX-C-042) was generated comprising an PMO ASO that targets exon 23 of DMD covalently linked to DTX-A-002 (RI7 217 (Fab)), an anti-transferrin receptor antibody.

[000552] Briefly, a Bicyclo[6.1.0]nonyne-PEG3-L-valine-L-citrulline-pentafluorophenyl ester (BCN-PEG3-Val-Cit-PFP) linker molecule was coupled to NH_2-C_6-(exon-23 PMO) using an amide coupling reaction. Excess linker and organic solvents were removed by gel permeation chromatography. The purified Val-Cit-linker-(exon-23 PMO) was then coupled to an azide functionalized anti-transferrin receptor antibody (DTX-A-002) generated through modifying ε-amine on lysine with Azide-PEG4-PFP.

[000553] The product of the antibody coupling reaction was then purified and densitometry confirmed that this sample of DTX-C-042 complexes had an average ASO to antibody ratio of 1.9.

[000554] The PMO ASO that targets exon 23 of DMD used in this Example comprises a sequence consisting of GGCCAAACCUCGGCUUACCUGAAAU (SEQ ID NO: 171).

[000555] DTX-C-042 was tested for its ability to induce exon skipping of exon 23 of the dystrophin gene, and to subsequently increase expression of dystrophin protein in targeted muscles relevant to DMD *in vivo*. *mdx* mice, a DMD mouse model, were intravenously injected with a single dose of a vehicle control (saline); DTX-C-042 complex at a dose of 10 mg/kg ASO; DTX-C-042 complex at a dose of 20 mg/kg ASO; or DTX-C-042 complex at a dose of 30 mg/kg ASO. Each experimental condition was replicated in four *mdx* mice. Four wild-type mice were also dosed with vehicle control (saline) as a control experiment.

[000556] Fourteen days after treatment, mice were euthanized and targeted muscle tissues were collected. Individual muscle tissue samples were subsequently assayed for percent skipping of exon 23 of the dystrophin gene (FIG. 5). Additionally, dystrophin protein levels in

targeted muscles were also quantified (quantification of dystrophin in quadriceps is shown in FIGs. 6A-6B).

[000557] Mice treated with the DTX-C-042 complex demonstrated a dose-dependent increase in the percent exon skipping of exon 23 in quadriceps, diaphragm, and heart muscles. Mice treated with the DTX-C-042 complex also demonstrated a dose-dependent increase in the expression of dystrophin protein in the quadriceps, with an average of >4% dystrophin protein in mice treated with 30 mg/kg ASO equivalent of DTX-C-042.

[000558] These data demonstrate that the anti-transferrin receptor antibody of the antibody-ASO complex enables cellular internalization of the complex into muscle-specific tissues in an *in vivo mdx* mouse model, thereby allowing the exon 23 PMO ASO to induce exon skipping of exon 23 of DMD. These data further demonstrate that the antibody-ASO complex is capable of specifically targeting muscle tissues.

Example 6: Targeting DMD with a muscle-targeting complex to demonstrate functional benefit in *mdx* mouse model

[000559] *Mdx* mice (DMD mouse model; diseased mice) were intravenously injected with a single dose of a vehicle control (saline); the MDX-ASO (naked exon 23 skipping PMO ASO, 30 mg/kg); or the DTX-C-042 complex as described in Example 5 (anti-transferrin receptor antibody RI7 217 Fab linked to exon 23 skipping PMO, 30 mg/kg ASO equivalent). Each experimental condition was replicated in five *mdx* mice. Five wild-type mice (healthy mice) were also dosed with vehicle control (saline).

[000560] Two and four weeks after injection, the functional activity of all treated mice was determined using an open field chamber experiment. The experiment involved three consecutive stages: (1) a 10-minute period during which each mouse was placed into an open field chamber; (2) a 10-minute period during which each mouse was subjected to a hind limb fatigue challenge; and (3) a 10-minute period during which each mouse was placed into an open field chamber. The total horizontal distances traveled during stages (1) and (3) were collected. The percent change in the total distance traveled between the first and second tests. As shown in FIG. 7A, at the two week timepoint, the wild-type mice treated with saline traveled an average of about 20% less during stage (3) relative to stage (1); the *mdx* mice treated with saline traveled an average of about 70% less during stage (3) relative to stage (1); the *mdx* mice treated with MDX-ASO traveled an average of about 85% less during stage (3) relative to stage (1); and the *mdx* mice treated with DTX-C-042 traveled an average of about 40% less during stage (3) relative to stage (1). When compared to wild-type mice treated with

saline, *mdx* mice treated with saline performed significantly worse (as indicated by a significant decrease in distance traveled in stage (3) relative to stage (1)). This observation is consistent with the impaired motor function experienced by DMD patients. *mdx* mice treated with naked MDX-ASO showed the same compromised functional performance as those treated with vehicle. In contrast, the performance of *mdx* mice treated with DTX-C-042 was not statistically different from vehicle treated wild-type mice. As shown in FIG. 7B, at the four week timepoint, the wild-type mice treated with saline traveled an average of about 35% less during stage (3) relative to stage (1); the *mdx* mice treated with saline traveled an average of about 80% less during stage (3) relative to stage (1); the *mdx* mice treated with MDX-ASO traveled an average of about 55% less during stage (3) relative to stage (1); and the *mdx* mice treated with DTX-C-042 traveled an average of about 50% less during stage (3) relative to stage (1).

[000561] Two and four weeks after injection, the activity of all treated mice was determined using a cage running wheel test. Each mouse was individually placed into cages with a running wheel for a 24-hour period. The 24-hour period involved five hours of light on followed by thirteen hours of darkness, and ending with six hours of light. The total distance traveled (in meters, m) by each mouse on the running wheel was continuously collected throughout the 24-hour period and subsequently binned into discrete one-hour increments. As shown in FIG. 7C, at the two week timepoint, the distance traveled by the *mdx* mice treated with DTX-C-042 was higher than the distance traveled by the *mdx* mice treated with MDX-ASO or with saline, and approached the distance traveled by the wild-type mice at certain times. As shown in FIG. 7D, at the four week timepoint, the distance traveled by the *mdx* mice treated with DTX-C-042 complex closely mirrored the total distance traveled by the wild-type mice treated with saline during the dark period (*i.e.*, when mice are active). This is in contrast to the *mdx* mice treated with either saline or MDX-ASO, which traveled considerably shorter distances during the dark period.

[000562] All mice in this Example were further tested for creatine kinase activity levels two weeks and four weeks after injection. Wild-type mice do not secrete large amounts of creatine kinase from muscle tissues. Conversely, *mdx* mice (having diseased muscle tissues) do secrete high levels of creatine kinase, which can be observed by determination of creatine kinase enzymatic activities. As shown in FIG. 7E, the *mdx* mice that were treated with saline had approximately 9- and 10- fold more creatine kinase enzymatic activity relative to wild-type mice treated with saline after two and four weeks, respectively. Dosing with naked ASO provided no significant benefit to the *mdx* mice. However, dosing *mdx* mice with DTX-C-042

complex did provide a statistically significant reduction in levels of creatine kinase activity after both two and four weeks.

[000563] These surprising results show that the antibody-ASO complex is capable of providing functional benefits to mice suffering from a DMD phenotype (*mdx* mice), such that these mice have phenotypic indicators resembling healthy (wild-type) mice. The performance of the antibody-ASO complex relative to the naked PMO (MDX-ASO) demonstrates that the anti-transferrin receptor antibody of the antibody-ASO complex is responsible for providing the functional benefits shown in this Example.

Example 7: Binding Affinity of selected anti-TfR1 antibodies to human TfR1

[000564] Selected anti-TfR1 antibodies were tested for their binding affinity to human TfR1 for measurement of Ka (association rate constant), Kd (dissociation rate constant), and K_D (affinity). Two known anti-TfR1 antibodies were used as control, 15G11 and OKT9. The binding experiment was performed on Carterra LSA at 25°C. An anti-mouse IgG and anti-human IgG antibody "lawn" was prepared on a HC30M chip by amine coupling. The IgGs were captured on the chip. Dilution series of hTfR1, cyTfR1, and hTfR2 were injected to the chip for binding (starting from 1000 nM, 1:3 dilution, 8 concentrations).

[000565] Binding data were referenced by subtracting the responses from a buffer analyte injection and globally fitting to a 1:1 Langmuir binding model for estimate of Ka (association rate constant), Kd (dissociation rate constant), and K_D (affinity) using the CarterraTM Kinetics software. 5-6 concentrations were used for curve fitting.

[000566] The result showed that the mouse mAbs demonstrated binding to hTfR1 with K_D values ranging from 13 pM to 50 nM. A majority of the mouse mAbs had K_D values in the single digit nanomolar to sub-nanomolar range. The tested mouse mAbs showed cross-reactive binding to cyTfR1 with K_D values ranging from 16 pM to 22 nM.

[000567] Ka, Kd, and K_D values of anti-TfR1 antibodies are provided in Table 7.

Table 7. Ka, Kd, and K_D values of anti-TfR1 antibodies

Name	K_D (M)	Ka (M)	Kd (M)
ctrl-15G11	2.83E-10	3.70E+05	1.04E-04
ctrl-OKT9 mIgG	5.36E-10	7.74E+05	4.15E-04
3-A04	4.36E-10	4.47E+05	1.95E-04
3-M12	7.68E-10	1.66E+05	1.27E-04
5-H12	2.08E-07	6.67E+04	1.39E-02

Example 8: Conjugation of anti-TfR1 antibodies with oligonucleotides

[000568] Complexes containing an anti-TfR1 antibody covalently conjugated to a tool oligo (ASO300) were generated. First, Fab fragments of anti-TfR antibody clones 3-A4, 3-M12, and 5-H12 were prepared by cutting the mouse monoclonal antibodies with an enzyme in or below the hinge region of the full IgG followed by partial reduction. The Fabs were confirmed to be comparable to mAbs in avidity or affinity.

[000569] Muscle-targeting complexes were generated by covalently linking the anti-TfR mAbs to the ASO300 via a cathepsin cleavable linker. Briefly, a Bicyclo[6.1.0]nonyne-PEG3-L-valine-L-citrulline-pentafluorophenyl ester (BCN-PEG3-Val-Cit-PFP) linker molecule was coupled to the ASO300 through a carbamate bond. Excess linker and organic solvents were removed by tangential flow filtration (TFF). The purified Val-Cit-linker-ASO was then coupled to an azide functionalized anti-transferrin receptor antibody generated through modifying ε-amine on lysine with Azide-PEG4-PFP. A positive control muscle-targeting complex was also generated using 15G11.

[000570] The product of the antibody coupling reaction was then subjected to two purification methods to remove free antibody and free payload. Concentrations of the conjugates were determined by either Nanodrop A280 or BCA protein assay (for antibody) and Quant-It Ribogreen assay (for payload). Corresponding drug-antibody ratios (DARs) were calculated. DARs ranged between 0.8 and 2.0, and were standardized so that all samples receive equal amounts of payload.

[000571] The purified complexes were then tested for cellular internalization and inhibition of the target gene, DMPK. Non-human primate (NHP) or DM1 (donated by DM1 patients) cells were grown in 96-well plates and differentiated into myotubes for 7 days. Cells were then treated with escalating concentrations (0.5 nM, 5 nM, 50 nM) of each complex for 72 hours. Cells were harvested, RNA was isolated, and reverse transcription was performed to generate cDNA. qPCR was performed using TaqMan kits specific for Ppib (control) and DMPK on the QuantStudio 7. The relative amounts of remaining DMPK transcript in treated vs non-treated cells were calculated and the results are shown in Table 12.

[000572] The results demonstrated that the anti-TfR1 antibodies are able to target muscle cells, be internalized by the muscle cells with the molecular payload (the tool oligo ASO300), and that the molecular payload is able to target and knockdown the target gene (DMPK). Knockdown activity of a complex comprising the anti-TfR1 antibody conjugated to a molecular payload (e.g., an oligonucleotide) targeting DMD may be tested using the same assay as described herein, e.g., by using any one of the oligonucleotides described in Table 14,

provided by any one of SEQ ID NO: 437-1241, or complementary to any one of SEQ ID NO: 1242-2046.

Table 12. Binding Affinity of anti-TfR1 Antibodies and Efficacy of Conjugates

Clone Name	huTfR1 Avg K_D (M) (antibody alone)	cyTfR1 Avg K_D (M) (antibody alone)	% knockdown of DMPK in NHP cells using Antibody-DMPK ASO conjugate	% knockdown of DMPK in cells from human DM1 patients using Antibody-DMPK ASO conjugate
15G11 (control)	8.0E-10	1.0E-09	36	46
3-A4	4.36E-10	2.32E-09	77	70
3-M12	7.68E-10	5.18E-09	77	52
5-H12	2.02316E-07	1.20E-08	88	57

[000573] Interestingly, the DMPK knockdown results showed a lack of correlation between the binding affinity of the anti-TfR to transferrin receptor and efficacy in delivering a DMPK ASO to cells for DMPK knockdown. Surprisingly, the anti-TfR antibodies provided herein (e.g., at least 3-A4, 3-M12, and 5-H12) demonstrated superior activity in delivering a payload (e.g., DMPK ASO) to the target cells and achieving the biological effect of the molecular payload (e.g., DMPK knockdown) in either cyno cells or human DM1 patient cells, compared to the control antibody 15G11, despite the comparable binding affinity (or, in certain instances, such as 5-H12, lower binding affinity) to human or cyno transferrin receptor between these antibodies and the control antibody 15G11.

[000574] Top attributes such as high huTfR1 affinity, >50% knockdown of DMPK in NHP and DM1 patient cell line, identified epitope binding with 3 unique sequences, low/no predicted PTM sites, and good expression and conjugation efficiency led to the selection of the top 3 clones for humanization, 3-A4, 3-M12, and 5-H12.

Example 9: Humanized anti-TfR1 antibodies

[000575] The anti-TfR antibodies shown in Table 2 were subjected to humanization and mutagenesis to reduce manufacturability liabilities. The humanized variants were screened and tested for their binding properties and biological actives. Humanized variants of anti-TfR1 heavy and light chain variable regions (5 variants each) were designed using Composite Human Technology. Genes encoding Fabs having these heavy and light chain variable regions were synthesized, and vectors were constructed to express each humanized heavy and light chain variant. Subsequently, each vector was expressed on a small scale and the resultant

humanized anti-TfR1 Fabs were analyzed. Humanized Fabs were selected for further testing based upon several criteria including Biocore assays of antibody affinity for the target antigen, relative expression, percent homology to human germline sequence, and the number of MHC class II predicted T cell epitopes (determined using iTope™ MCH class II in silico analysis).

[000576] Potential liabilities were identified within the parental sequence of some antibodies by introducing amino acid substitutions in the heavy chain and light chain variable regions. These substitutions were chosen based on relative expression levels, iTope™ score and relative K_D from Biacore single cycle kinetics analysis. The humanized variants were tested and variants were selected initially based upon affinity for the target antigen. Subsequently, the selected humanized Fabs were further screened based on a series of biophysical assessments of stability and susceptibility to aggregation and degradation of each analyzed variant, shown in **Table 16** and **Table 17**. The selected Fabs were analyzed for their properties binding to TfR1 by kinetic analysis. The results of these analyses are shown in **Table 13**. For conjugates shown in Table 16 and Table 17, the selected humanized Fabs were conjugated to a DMPK-targeting oligonucleotide ASO300. The selected Fabs are thermally stable, as indicated by the comparable binding affinity to human and cyno TfR1 after been exposed to high temperature (40 °C) for 9 days, compared to before the exposure (see Table 13).

Table 16. Biophysical assessment data for humanized anti-TfR Fabs

Variant / Criteria	3M12 (VH3/Vk2)	3M12 (VH3/Vk3)	3M12 (VH4/Vk2)	3M12 (VH4/Vk3)	3A4 (VH3-N54T/Vk4)
Binding Affinity (Biacore d0)	395 pM	345 pM	396 pM	341 pM	3.09 nM
Binding Affinity (Biacore d25)	567 pM	515 pM	510 pM	486 pM	3.01 nM
Fab binding affinity ELISA (human/cyno TfR1)	0.8 nM/9.9 nM	0.6 nM/4.7 nM	0.4 nM/1.4 nM	0.5 nM/2.2 nM	2.6 nM/156 nM*
Conjugate binding affinity ELISA (human/cyno TfR1)	2.2 nM/2.9 nM	N/A	N/A	1.7 nM/2.1 nM	2.8 nM/4.7 nM
...					
Variant / Criteria	**3A4 (VH3-N54S/Vk4)**	**3A4 (VH3/Vk4)**	**5H12 (VH5-C33Y/Vk3)**	**5H12 (VH5-C33D/Vk4)**	**5H12 (VH4-C33Y/Vk4)**
Binding Affinity (Biacore d0)	1.34 nM	1.5 nM	627 pM	991 pM	626 pM
Binding Affinity (Biacore d25)	1.39 nM	1.35 nM	1.07 nM	3.01 nM	1.33 nM
Fab binding affinity ELISA (human/cyno TfR1)	1.6 nM/398 nM*	1.5 nM/122 nM*	6.3 nM/2.1 nM	6.0 nM/3.5 nM	2.8 nM/3.3 nM
Conjugate binding affinity ELISA (human/cyno TfR1)	2.9 nM/7.8 nM	2.8 nM/7.6 nM	33.4 nM/2.3 nM	110 nM/10.2 nM	23.7 nM/3.3 nM

*Regains cyno binding after conjugation;

Table 17. Thermal Stability for humanized anti-TfR Fabs and conjugates

Variant / Criteria	3M12 (VH3/Vk2)	3M12 (VH3/Vk3)	3M12 (VH4/Vk2)	3M12 (VH4/Vk3)	3A4 (VH3-N54T/Vk4)
Binding affinity hTfR1 d0 (nM)	0.8	0.6	0.4	0.5	2.6
Binding affinity hTfR1 d9 (nM)	0.98	1.49	0.50	0.28	0.40
Binding affinity cyno TfR1 d0 (nM)	9.9	4.7	1.4	2.2	156
Binding affinity cyno TfR1 d9 (nM)	19.51	15.58	5.01	16.40	127.50
DMPK oligo conjugate binding to hTfR1 (nM)	1.14	N/A	N/A	1.18	2.22
DMPK oligo conjugate binding to cyno TfR1 (nM)	2.26	N/A	N/A	1.85	5.12
...					
Variant / Criteria	**3A4 (VH3-N54S/Vk4)**	**3A4 (VH3/Vk4)**	**5H12 (VH5-C33Y/Vk3)**	**5H12 (VH5-C33D/Vk4)**	**5H12 (VH4-C33Y/Vk4)**
Binding affinity hTfR1 d0 (nM)	1.6	1.5	6.3	6	2.8
Binding affinity hTfR1 d9 (nM)	0.65	0.46	71.90	92.34	1731.00
Binding affinity cyno TfR1 d0 (nM)	398	122	2.1	3.5	3.3
Binding affinity cyno TfR1 d9 (nM)	248.30	878.40	0.69	0.63	0.26
DMPK oligo conjugate binding to hTfR1 (nM)	2.71	2.837	N/A	110.5	13.9
DMPK oligo conjugate binding to cyno TfR1 (nM)	4.1	7.594	N/A	10.18	13.9

Table 13. Kinetic analysis of humanized anti-TfR Fabs binding to TfR1

Humanized anti-TfR Fabs	k_a (1/Ms)	k_d (1/s)	K_D (M)	R_{MAX}	Chi^2 (RU^2)
3A4 (VH3/Vk4)	7.65E+10	1.15E+02	1.50E-09	48.0	0.776
3A4 (VH3-N54S/Vk4)	4.90E+10	6.56E+01	1.34E-09	49.4	0.622
3A4 (VH3-N54T/Vk4)	2.28E+05	7.05E-04	3.09E-09	61.1	1.650
3M12 (VH3/Vk2)	2.64E+05	1.04E-04	3.95E-10	78.4	0.037
3M12 (VH3/Vk3)	2.42E+05	8.34E-05	3.45E-10	91.1	0.025
3M12 (VH4/Vk2)	2.52E+05	9.98E-05	3.96E-10	74.8	0.024
3M12 (VH4/Vk3)	2.52E+05	8.61E-05	3.41E-10	82.4	0.030
5H12 (VH5-C33D/Vk4)	6.78E+05	6.72E-04	9.91E-10	49.3	0.093
5H12 (VH5-C33Y/Vk3)	1.95E+05	1.22E-04	6.27E-10	68.5	0.021
5H12 (VH5-C33Y/Vk4)	1.86E+05	1.17E-04	6.26E-10	75.2	0.026

Binding of humanized anti-TfR1 Fabs to TfR1 (ELISA)

[000577] To measure binding of humanized anti-TfR antibodies to TfR1, ELISAs were conducted. High binding, black, flat bottom, 96 well plates (Corning# 3925) were first coated

with 100 µL/well of recombinant huTfR1 at 1 µg/mL in PBS and incubated at 4°C overnight. Wells were emptied and residual liquid was removed. Blocking was conducted by adding 200 µL of 1%BSA (w/w) in PBS to each well. Blocking was allowed to proceed for 2 hours at room temperature on a shaker at 300 rpm. After blocking, liquid was removed and wells were washed three times with 300 µL of TBST. Anti-TfR1 antibodies were then added in 0.5% BSA/TBST in triplicate in an 8 point serial dilution (dilution range 5 µg/mL – 5 ng/mL). A positive control and isotype controls were also included on the ELISA plate. The plate was incubated at room temperature on an orbital shaker for 60 minutes at 300 rpm, and the plate was washed three times with 300 µL of TBST. Anti-(H+L)IgG-A488 (1:500) (Invitrogen #A11013) was diluted in 0.5% BSA in TBST, and 100 µL was added to each well. The plate was then allowed to incubate at room temperature for 60 minutes at 300 rpm on orbital shaker. The liquid was removed and the plate was washed four times with 300 µL of TBST. Absorbance was then measured at 495 nm excitation and 50 nm emission (with a 15 nm bandwidth) on a plate reader. Data was recorded and analyzed for EC_{50}. The data for binding to human TfR1 (hTfR1) for the humanized 3M12, 3A4 and 5H12 Fabs are shown in **FIG. 9A**, **9C**, and **9E**, respectively. ELISA measurements were conducted using cynomolgus monkey (*Macaca fascicularis*) TfR1 (cTfR1) according to the same protocol described above for hTfR1, and results are shown in **FIG. 9B**, **9D**, and **9F**.

[000578] Results of these two sets of ELISA analyses for binding of the humanized anti-TfR Fabs to hTfR1 and cTfR1 demonstrate that humanized 3M12 Fabs show consistent binding to both hTfR1 and cTfR1, and that humanized 3A4 Fabs show decreased binding to cTfR1 relative to hTfR1.

[000579] Antibody-oligonucleotide conjugates were prepared using six humanized anti-TfR Fabs, each of which were conjugated to a DMPK targeting oligonucleotide ASO300. Conjugation efficiency and down-stream purification were characterized, and various properties of the product conjugates were measured. The results demonstrate that conjugation efficiency was robust across all 10 variants tested, and that the purification process (hydrophobic interaction chromatography followed by hydroxyapatite resin chromatography) were effective. The purified conjugates showed a >97% purity as analyzed by size exclusion chromatography.

[000580] Several humanized Fabs were tested in cellular uptake experiments to evaluate TfR1-mediated internalization. To measure such cellular uptake mediated by antibodies, humanized anti-TfR Fab conjugates were labeled with Cypher5e, a pH-sensitive dye. Rhabdomyosarcoma (RD) cells were treated for 4 hours with 100 nM of the conjugates,

trypsinized, washed twice, and analyzed by flow cytometry. Mean Cypher5e fluorescence (representing uptake) was calculated using Attune NxT software. As shown in FIG. 10, the humanized anti-TfR Fabs show similar or greater endosomal uptake compared to a positive control anti-TfR1 Fab. Similar internalization efficiencies were observed for different oligonucleotide payloads. An anti-mouse TfR antibody was used as the negative control. Cold (non-internalizing) conditions abrogated the fluorescence signal of the positive control antibody-conjugate (data not shown), indicating that the positive signal in the positive control and humanized anti-TfR Fab-conjugates is due to internalization of the Fab-conjugates. Similarly, an oligonucleotide that induces DMD exon skipping can also be conjugated the humanized anti-TfR Fabs for cellular uptake by muscle cells.

[000581] Conjugates of six humanized anti-TfR Fabs of were also tested for binding to hTfR1 and cTfR1 by ELISA, and compared to the unconjugated forms of the humanized Fabs. Results demonstrate that humanized 3M12 and 5H12 Fabs maintain similar levels of hTfR1 and cTfR1 binding after conjugation relative to their unconjugated forms (3M12, **FIG. 11A** and **11B**; 5H12, **FIG. 11E** and **11F**). Interestingly, 3A4 clones show improved binding to cTfR1 after conjugation relative to their unconjugated forms (**FIG. 11C** and **11D**).

[000582] As used in this Example, the term 'unconjugated' indicates that the antibody was not conjugated to an oligonucleotide.

Example 10. Knockdown of DMPK mRNA level facilitated by antibody-oligonucleotide conjugates *in vitro*

[000583] Conjugates containing humanized anti-TfR Fabs 3M12(VH3/Vk2), 3M-12 (VH4/Vk3), and 3A4(VH3-N54S/Vk4) were conjugated to a DMPK-targeting oligonucleotide ASO300 and were tested in rhabdomyosarcoma (RD) cells for knockdown of DMPK transcript expression. Antibodies were conjugated to ASO300 via the linker shown in Formula (C).

[000584] RD cells were cultured in a growth medium of DMEM with glutamine, supplemented with 10% FBS and penicillin/streptomycin until nearly confluent. Cells were then seeded into a 96 well plate at 20K cells per well and were allowed to recover for 24 hours. Cells were then treated with the conjugates for 3 days. Total RNA was collected from cells, cDNA was synthesized and DMPK expression was measured by qPCR.

[000585] Results in FIG. 12 show that DMPK expression level was reduced in cells treated with each indicated conjugate, relative to expression in PBS-treated cells, indicating that the humanized anti-TfR Fabs are able to mediate the uptake of the DMPK-targeting

oligonucleotide by the RD cells and that the internalized DMPK-targeting oligonucleotide are effective in knocking down DMPK mRNA level.

[000586] Similarly, an oligonucleotide that induces DMD exon skipping can also be conjugated the humanized anti-TfR Fabs for delivery to muscle cells and inducing DMD exon skipping in muscle cells.

Example 11. Anti-TfR-oligonucleotide conjugate treatment increased dystrophin expression in *mdx* mouse model of DMD

[000587] To test the effects of another oligonucleotide that induces DMD exon skipping *in vivo*, an oligonucleotide (PMO) that induces exon 23 skipping was administered as naked oligonucleotide or in conjugate with an anti-mouse TfR antibody to the *mdx* mouse model of DMD. Dystrophin expression was measured. The exon skipping promoted by the conjugate resulted in dose-dependent production of dystrophin protein as illustrated by western blot (**FIG. 14**) and quantified in **FIG. 15**. Alpha-actin was used as a loading control.

[000588] A single dose of the exon 23-conjugate administered in the *mdx* mouse also restored dystrophin expression to the muscle cell membrane in addition to increasing overall dystrophin levels, as shown in **FIG. 16**. Immunofluorescence staining of dystrophin in quadricep muscles demonstrated that *mdx* mice treated with the conjugated had higher levels of dystrophin in their quadriceps than mice treated with naked oligonucleotide or saline.

Example 12. Oligonucleotide-mediated exon skipping in DMD myotubes

[000589] Promoting the skipping of specific DMD exons in the nucleus could allow muscle cells to create more complete, functional dystrophin protein. An oligonucleotide (PMO) that induces skipping of DMD exon 51 was conjugated to an anti-TfR1 Fab and the conjugated was tested in human DMD myotubes with a mutation amenable to Exon 51 skipping. Treatment with the conjugate resulted in a 50% increase in exon skipping as compared to a 25% increase in exon skipping following treatment with an equimolar dose of the naked oligonucleotide (p-value=0.001), as shown in FIG. 13. Similar results were observed in the *mdx* mouse model of DMD, such as those shown in FIG. 5.

Example 13. Serum stability of the linker linking the anti-TfR antibody and the molecular payload

[000590] Oligonucleotides which were linked to antibodies in examples were conjugated via a cleavable linker shown in Formula (C). It is important that the linker maintain stability in

serum and provide release kinetics that favor sufficient payload accumulation in the targeted muscle cell. This serum stability is important for systemic intravenous administration, stability of the conjugated oligonucleotide in the bloodstream, delivery to muscle tissue and internalization of the therapeutic payload in the muscle cells. The linker has been confirmed to facilitate precise conjugation of multiple types of payloads (including ASOs, siRNAs and PMOs) to Fabs. This flexibility enabled rational selection of the appropriate type of payload to address the genetic basis of each muscle disease. Additionally, the linker and conjugation chemistry allowed the optimization of the ratio of payload molecules attached to each Fab for each type of payload, and enabled rapid design, production and screening of molecules to enable use in various muscle disease applications.

[000591] FIG. 8 shows serum stability of the linker *in vivo*, which was comparable across multiple species over the course of 72 hours after intravenous dosing. At least 75% stability was measured in each case at 72 hours after dosing.

Example 14. Exon-skipping activity of anti-TfR conjugates in DMD patient myotubes

[000592] In this study, the exon-skipping activities of anti-TfR conjugates containing an anti-TfR Fab (3M12 VH3/Vκ2, 3M12 VH4/Vκ3, and 3A4 VH3 N54S/Vκ4) conjugated to a DMD exon 51-skipping oligonucleotide were evaluated. Immortalized human myoblasts bearing an exon 52 deletion were thawed and seeded at a density of 1e6 cell/flask in Promocell Skeletal Cell Growth Media (with 5% FBS and 1x Pen-Strep) and allowed to grow to confluency. Once confluent, cells were trypsinized and pelleted via centrifugation and resuspended in fresh Promocell Skeletal Cell Growth Media. The cell number was counted and cells were seeded into Matrigel-coated 96-well plates at a density of 50k cells/well. Cells were allowed to recover for 24 hours. Cells were induced to differentiate by aspirating the growth media and replacing with differentiation media with no serum. Cells were then treated with conjugated or unconjugated DMD exon skipping oligonucleotide at 10 μM. Cells were incubated with test articles for ten days then total RNA was harvested from the 96 well plates. cDNA synthesis was performed on 75 ng of total RNA, and mutation specific PCRs were performed to evaluate the degree of exon 51 skipping in each cell type. Mutation-specific PCR products were run on a 4% agarose gel and visualized using SYBR gold. Densitometry was used to calculate the relative amounts of the skipped and unskipped amplicon and exon skipping was determined as a ratio of the Exon 51 skipped amplicon divided by the total amount of amplicon present: $\%Exon\ Skipping = \frac{Skipped\ Amplicon}{(Skipped\ Amplicon + Unskipped\ Amplicon)} * 100$

[000593] The results demonstrate that the conjugates with either 3M12 VH3/Vκ2 or 3M12 VH4/Vκ3 Fab covalently linked to the DMD exon 51-skipping oligonucleotide resulted in enhanced exon skipping compared to the unconjugated DMD exon skipping oligonucleotide in patient myotubes (FIG. 17).

[000594] As used in this Example, the term 'unconjugated' indicates that the oligonucleotide was not conjugated to an antibody.

Example 15. Characterization of binding activities of anti-TfR Fab 3M12 VH4/Vk3

[000595] In vitro studies were performed to test the specificity of anti-TfR Fab 3M12 VH4/Vκ3 for human and cynomolgus monkey TfR1 binding and to confirm its selectivity for human TfR1 vs TfR2. The binding affinity of anti-TfR Fab 3M12 VH4/Vκ3 to TfR1 from various species was determined using an enzyme-linked immunosorbent assay (ELISA). Serial dilutions of the Fab were added to plates precoated with recombinant human, cynomolgus monkey, mouse, or rat TfR1. After a short incubation, binding of the Fab was quantified by addition of a fluorescently tagged anti-(H+L) IgG secondary antibody and measurement of fluorescence intensity at 495nm excitation and 520nm emission. The Fab showed strong binding affinity to human and cynomolgus monkey TfR1, and no detectable binding of mouse or rat TfR1 was observed (FIG. 18). Surface plasmon resonance (SPR) measurements were also conducted, and results are shown in Table 18. The K_d of the Fab against the human TfR1 receptor was calculated to be 7.68×10^{-10} M and against the cynomolgus monkey TfR1 receptor was calculated to be 5.18×10^{-9} M.

Table 18. Kinetic analysis of anti-TfR Fab 3M12 VH4/Vk3 binding to human and cynomolgus monkey TfR1 or human TfR2, measured using surface plasmon resonance

	Anti-TfR Fab 3M12 VH4/Vk3				
Target	K_d (M)	k_a (M^{-1} s^{-1})	k_d (s^{-1})	R_{max}	R_{es} SD
Human TfR1	7.68E-10	1.66E+05	1.27E-04	1.11E+02	3.45E+00
Cyno TfR1	5.18E-09	9.19E+04	4.76E-04	1.87E+02	6.24E+00
Human TfR2	ND	ND	ND	ND	ND

ND = No detectable binding by SPR (10pM – 100 uM)

[000596] To test for cross-reactivity of anti-TfR Fab 3M12 VH4/Vk3 to human TfR2, an ELISA was performed. Recombinant human TfR2 protein was plated overnight at 2 μg/mL and was blocked for 1 hour with 1% bovine serum albumin (BSA) in PBS. Serial dilutions of the Fab or a positive control anti-TfR2 antibody were added in 0.5% BSA/TBST for 1 hour. After washing, anti-(H+L) IgG-A488 (Invitrogen #MA5-25932) fluorescent secondary antibody was added at a 1:500 dilution in 0.5% BSA/TBST and the plate was incubated for 1 hour. Relative fluorescence was measured using a Biotek Synergy plate reader at 495nm

excitation and 520nm emission. No binding of anti-TfR Fab 3M12 VH4/Vk3 to hTfR2 was observed (FIG. 19).

Example 16. Serum stability of anti-TfR Fab-ASO conjugate

[000597] Anti-TfR Fab VH4/Vk3 was conjugated to a control antisense oligonucleotide (ASO) via a linker as shown in Formula (C) and the resulting conjugate was tested for stability of the linker conjugating the Fab to the ASO. Serum stability was measured by incubating fluorescently labeled conjugate in PBS or in rat, mouse, cynomolgus monkey, or human serum and measuring relative fluorescence intensity over time, with higher fluorescence indicating more conjugate remaining intact. FIG. 20 shows serum stability was similar across multiple species and remained high after 72 hours.

Example 17. Exon skipping activity of anti-TfR Fab-ASO conjugate in vivo in cynomolgus monkeys

[000598] Anti-TfR Fab 3M12 VH4/Vk3 was conjugated to a dystrophin (DMD) exon 51-skipping antisense oligonucleotide (ASO) targeting an ESE as set forth in SEQ ID NO: 419. The exon 51 skipping oligonucleotide is a phosphorodiamidate morpholino oligomer (PMO) of 30 nucleotides in length. The exon skipping activity of the conjugate was tested in vivo in healthy non-human primates. Naïve male cynomolgus monkeys (n= 4-5 per group) were administered two doses of vehicle, 30 mg/kg ASO alone, or 122 mg/kg conjugate (30 mg/kg ASO equivalent) via intravenous infusion on days 1 and 8. Animals were sacrificed and tissues harvested either 2 weeks or 4 weeks after the first dose was administered. Total RNA was collected from tissue samples using a Promega Maxwell® RSC instrument and cDNA synthesis was performed using qScript cDNA SuperMix. Assessment of exon 51 skipping was performed using end-point PCR.

[000599] Capillary electrophoresis of the PCR products was used to assess exon skipping, and % exon 51 skipping was calculated using the following formula:

$$\%\ Exon\ Skipping = \frac{Molarity\ of\ Skipped\ Band}{Molarity\ of\ Skipped\ Band + Molarity\ of\ Unskipped\ Band} * 100.$$

Calculated exon 51 skipping results are shown in Table 19.

Table 19. Exon 51 skipping of dystrophin in cynomolgus monkey dystrophin

Time	2 weeks			4 weeks	
Group	Vehicle	ASO alone[a]	Conjugate	ASO alone[a]	Conjugate
Conjugate dose[b]	0	n/a	122	n/a	122
ASO alone Dose[c]	0	30	30	30	30

Quadriceps [d]	0.00 (0.00)	1.216 (1.083)	4.906 (3.131)	0.840 (1.169)	1.708 (1.395)
Diaphragm [d]	0.00 (0.00)	1.891 (2.911)	7.315 (1.532)	0.717 (1.315)	9.225 (4.696)
Heart [d]	0.00 (0.00)	0.043 (0.096)	3.42 (1.192)	0.00 (0.00)	4.525 (1.400)
Biceps [d]	0.00 (0.00)	0.607 (0.615)	3.129 (0.912)	1.214 (1.441)	4.863 (3.881)
Tibialis anterior [d]	0.00 (0.00)	0.699 (0.997)	1.042 (0.685)	0.384 (0.615)	0.816 (0.915)
Gastrocnemius [d]	0.00 (0.00)	0.388 (0.573)	2.424 (2.329)	0.00 (0.00)	5.393 (2.695)

[a]ASO = antisense oligonucleotide.
[b]Conjugate doses are listed as mg/kg of anti-TfR Fab 3M12 VH4/Vk3-ASO conjugate.
[c]ASO doses are listed as mg/kg ASO equivalent of the anti-TfR Fab 3M12 VH4/Vk3-ASO dose.
[d]Exon skipping values are mean % exon 51 skipping with standard deviations (n=5) in parentheses.

[000600] Tissue ASO accumulation was also quantified using a hybridization ELISA with a probe complementary to the ASO sequence. A standard curve was generated and ASO levels (in ng/g) were derived from a linear regression of the standard curve. The ASO was distributed to all tissues evaluated at a higher level following the administration of the anti-TfR Fab VH4/Vk3-ASO conjugate as compared to the administration of unconjugated ASO. Intravenous administration of unconjugated ASO resulted in levels of ASO that were close to background levels in all tissues evaluated at 2 and 4 weeks after the first does was administered. Administration of anti-TfR Fab VH4/Vk3-ASO conjugate resulted in distribution of ASO through the tissues evaluated with a rank order of heart>diaphragm>bicep>quadriceps>gastrocnemious>tibialis anterior 2 weeks after first dosing. The duration of tissue concentration was also assessed. Concentrations of the ASO in quadriceps, bicep and diaphragm decreased by less than 50% over the time period evaluated (2 to 4 weeks), while levels of ASO in the heart, tibialis anterior, and gastrocnemius remained virtually unchanged (Table 20).

[000601] As used in this Example, the term 'unconjugated' indicates that the oligonucleotide was not conjugated to an antibody.

Table 20. Tissue distribution of DMD exon 51 skipping ASO in cynomolgus monkeys

Time	**2 weeks**			**4 weeks**	
Group	**Vehicle**	**ASO alone[a]**	**Conjugate**	**ASO alone[a]**	**Conjugate**
Conjugate Dose[b]	0	n/a	122	n/a	122
ASO alone Dose[c]	0	30	30	30	30
Quadriceps [d]	0 (59.05)	696.8 (868.15)	2436 (954.0)	197 (134)	682 (281)
Diaphragm [d]	0± (144.3)	580.02 (360.11)	6750 (2256)	60 (120)	3131 (1618)
Heart [d]	0 (396.03)	1449 (1337)	27138 (6315)	943 (1803)	30410 (9247)

Biceps [d]	0 (69.58)	615.63 (335.17)	2840 (980.31)	130 (80)	1326 (623)
Tibialis anterior [d]	0 (76.31)	564.71 (327.88)	1591 (253.50)	169 (110)	1087 (514)
Gastrocnemius [d]	0 (41.15)	705.47 (863.75)	2096 (474.04)	170 (69)	1265 (272)

[a]ASO = Antisense oligonucleotide.
[b]Conjugate doses are listed as mg/kg of anti-TfR Fab 3M12 VH4/Vk3-ASO conjugate.
[c]ASO doses are listed as mg/kg ASO or ASO equivalent of the anti-TfR Fab 3M12 VH4/Vk3-ASO conjugate dose.
[d]ASO values are mean concentrations of ASO in tissue as ng/g with standard deviations (n=5) in parentheses.

Example 18. Effect of conjugates containing an anti-TfR1 Fab conjugated to an oligonucleotide that induces DMD exon 23 skipping on biomarker expression and muscle function in *mdx* mice

[000602] The objective of this study was to determine the effect of a single dose of anti-mouse TfR Fab conjugated to an antisense oligonucleotide (Ab-ASO) or of a single dose of the same naked ASO on dystrophin expression and muscle function in *mdx* mice. The complex used in this example was DTX-C-042 as described in Example 5.

[000603] Seven-week-old male *mdx* homozygous mice were allocated randomly to each of eight treatment groups. The mice were administered via tail vein a singled dose of ASO at 30 mg/kg, Ab-ASO at a dose equivalent to 30 mg/kg of ASO, or saline. Tissues were harvested and analyzed 2 weeks or 4 weeks following administration.

[000604] *Measurement of exon 23 skipping in muscles:* Quantification of exon 23 skipping was performed using a single-step RT-PCR reaction using the SuperScript® III (Thermo Fisher) with 75 ng total RNA input. The PCR primers used were 5'-CACATCTTTGATGGTGTGAGG (forward) (SEQ ID NO: 2264) and 5'-CAACTTCAGCCATCCATTTCTG (reverse) (SEQ ID NO: 2253). Capillary electrophoresis was used to quantitate the skipped and unskipped bands in the skeletal muscles of interest using the following equation:

$$\%\ Exon\ 23\ Skipping = \frac{Skipped\ Band}{Skipped\ Band + Unskipped\ Band} x\ 100$$

. The results demonstrate that a single administration of anti-TfR Fab-oligonucleotide conjugate (Ab-ASO) facilitated significant increases in skipping of exon 23 in quadriceps (FIG. 21A), heart (FIG. 21B), and diaphragm (FIG. 21C) of *mdx* mice. By contrast, little or no exon 23 skipping was observed in the same muscle tissues in wild-type (WT) mice or in *mdx* mice treated with saline or naked ASO.

[000605] *Measurement of dystrophin protein in muscles:* Muscle tissue samples taken from the quadriceps were homogenized and protein concentrations were measured by BCA

assay. Total protein (25 μg) was loaded onto a 3% to 8% Tris-acetate protein gel and run at 150 V for 1 hour. The gel was then transferred to a polyvinylidene difluoride membrane, and the membrane was cut, and the portion containing dystrophin was incubated overnight in anti-dystrophin antibody (Abcam catalog # 15277) at 4°C, followed by goat anti-rabbit IgG (H+L) horseradish peroxidase conjugate (Bio-Rad) for 30 minutes at room temperature. As a control, the remaining portion of the blot was incubated overnight with anti-alpha-actinin antibody (Abcam catalog # 9465) at 4°C, followed by goat anti-mouse IgG (H+L) horseradish peroxidase conjugate (Bio-Rad) for 15 minutes at room temperature. The blot was developed using the ECL Western Detection Kit (Cytiva) and quantified using iBright analysis software (Thermo Fisher Scientific). Images of Western blots are shown for muscle tissues collected two-weeks following injections in FIGs. 22A (quadriceps), 23A (heart), and 24A (diaphragm), and quantification of the Western blot results are shown in FIGs. 22B (quadriceps), 23B (heart), and 24B (diaphragm). Images of western blots are shown for muscle tissues collected four-weeks following injections in FIGs. 22C (quadriceps), 23C (heart), and 25C (diaphragm), and quantification of the Western blot results are shown in FIGs. 22D (quadriceps), 23D (heart), and 24D (diaphragm). In each western blot, the standard curve was generated using pooled protein from wild-type and *mdx* tissues, and the percentage wild-type (% WT) protein in each standard indicates the amount of wild-type protein in the sample. FIGs. 22A-22D demonstrate that two- and four-weeks following administration, Ab-ASO facilitated increases in dystrophin protein in quadriceps to a greater extent than unconjugated ASO. FIGs. 23A-23D demonstrate that two- and four-weeks following administration, Ab-ASO facilitated increases in dystrophin protein in heart muscle, whereas little to no wild-type dystrophin was measured in heart muscle from mice treated with naked ASO. FIGs. 24A-24D demonstrate that two- and four-weeks following administration, Ab-ASO facilitated increases in dystrophin protein in diaphragm muscle, whereas little to no wild-type dystrophin was measured in diaphragm muscle from mice treated with naked ASO.

[000606] *Measurement of ASO content within tissues*: Enzyme-linked immunosorbent assay (ELISA) was performed by coating NeutrAvidin coated plates with a capture probe specific to the ASO of interest. Proteinase K digested tissue lysate was incubated on the coated plates to allow binding of the ASO of interest to the capture probe. Plates were then washed and unbound capture probe was digested with micrococcal nuclease, followed by further washing and blocking. A Digoxigenin HRP-conjugated antibody was added to bind to intact capture probe, then imaged using TMB substrate (R&D Systems, Inc.). Quantification was performed using a standard curve of known concentration diluted into skeletal muscle matrix.

The results demonstrate that administration of the Fab conjugate is able to achieve substantial accumulation of ASO within quadriceps (FIG. 25A), diaphragm (FIG. 25B), and heart (FIG. 25C), whereas administration of naked ASO showed little or no ASO content in each muscle tissue. These results demonstrate that little or no ASO was detected in muscle tissues of mice administered saline or naked ASO, whereas administration of Ab-ASO resulted in measurable quantities of ASO in each of the tissues tested two- and four-weeks following administration.

Example 19. Conjugation of DMD exon 53-skipping oligonucleotides to anti-TfR1 antibodies improves their potency

[000607] To test the effect of anti-TfR1 targeting on exon 53-skipping oligonucleotides, complexes were formed comprising an anti-TfR1 Fab antibody (3M12 VH4/Vk3) covalently linked to exon 53-skipping PMOs via a linker having the structure of formula (C). Two exon 53-skipping PMOs were used in this Example: exon 53 PMO-A, comprising the sequence GTTGCCTCCGGTTCTGAAGGTGTTC (SEQ ID NO: 2256), and exon 53 PMO-B, comprising the sequence CCTCCGGTTCTGAAGGTGTTC (SEQ ID NO: 2257).

[000608] First, the exon 53-skipping PMOs alone were tested for their ability to facilitate skipping of exon 53 following gymnotic uptake (*i.e.*, without transfection agent or modification to confer muscle targeting). KM1328 DMD patient cells which harbor a deletion of DMD exon 52 were treated with a range of concentrations of exon 53 PMO-A or exon 53 PMO-B, and exon 53 skipping was measured. As shown in FIG. 26, exon 53 PMO-A was about 2-fold more potent than exon 53 PMO-B. Based on the dose response curves, it was calculated that a concentration of 2.5 μM of exon 53 PMO-A or 4.7 μM of exon 53 PMO-B is required to achieve 50% skipping of exon 53.

[000609] Next, complexes comprising the anti-TfR1 Fab covalently linked to either exon 53 PMO-A or exon 53 PMO-B ("anti-TfR1 Fab-ASO complex") were tested for their ability to facilitate skipping of exon 53 in KM1328 DMD patient cells in comparison with the same PMOs not linked to an antibody ("naked ASO"). Cells were treated with the naked ASO at concentrations of 0.16 μM, 0.32 μM, 0.63 μM, or 1.25 μM, or with the anti-TfR1 Fab-ASO complex at ASO equivalent concentrations of 0.16 μM, 0.32 μM, 0.63 μM, or 1.25 μM. As shown in FIG. 27, the Fab-ASO complexes achieved greater exon 53 skipping than did the naked ASO at each of the tested concentrations, including achieving significantly improved exon 53 skipping by exon 53 PMO-A at the lower doses tested (0.16 μM, 0.32 μM, and 0.63 μM). These results demonstrate that covalently linking exon-skipping oligonucleotides to anti-

TfR1 antibodies can facilitate exon-skipping activity at lower doses, thereby enabling efficacy of the oligonucleotides at lower doses.

ADDITIONAL EMBODIMENTS

1. A complex comprising a muscle-targeting agent covalently linked to a molecular payload configured for promoting the expression or activity of a DMD gene, wherein the muscle-targeting agent specifically binds to an internalizing cell surface receptor on muscle cells, wherein the muscle targeting agent is a humanized antibody, wherein the antibody comprises:

(i) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 69; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 70;

(ii) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 71; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 70;

(iii) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 72; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 70;

(iv) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 73; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 74;

(v) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 73; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 75;

(vi) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 76; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 74;

(vii) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 76; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 75;

(viii) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 77; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 78;

(ix) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 79; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 80; or

(x) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 77; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 80.

2. The complex of embodiment 1, wherein the antibody comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 69 and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(ii) a VH comprising the amino acid sequence of SEQ ID NO: 71and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(iii) a VH comprising the amino acid sequence of SEQ ID NO: 72 and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(iv) a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of SEQ ID NO: 74;

(v) a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of SEQ ID NO: 75;

(vi) a VH comprising the amino acid sequence of SEQ ID NO: 76 and a VL comprising the amino acid sequence of SEQ ID NO: 74;

(vii) a VH comprising the amino acid sequence of SEQ ID NO: 76 and a VL comprising the amino acid sequence of SEQ ID NO: 75;

(viii) a VH comprising the amino acid sequence of SEQ ID NO: 77 and a VL comprising the amino acid sequence of SEQ ID NO: 78;

(ix) a VH comprising the amino acid sequence of SEQ ID NO: 79 and a VL comprising the amino acid sequence of SEQ ID NO: 80; or

(x) a VH comprising the amino acid sequence of SEQ ID NO: 77 and a VL comprising the amino acid sequence of SEQ ID NO: 80.

3. The complex of embodiment 1 or embodiment 2, wherein the antibody is selected from the group consisting of a full-length IgG, a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a scFv, and a Fv.

4. The complex of embodiment 3, wherein the antibody is a full-length IgG, optionally wherein the full-length IgG comprises a heavy chain constant region of the isotype IgG1, IgG2, IgG3, or IgG4.

5. The complex of embodiment 4, wherein the antibody comprises:

(i) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 84; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(ii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 86; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(iii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 87; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(iv) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 88; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 89;

(v) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 88; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 90;

(vi) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 91; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 89;

(vii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 91; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 90;

(viii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 92; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 93;

(ix) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 94; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 95; or

(x) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 92; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 95.

6. The complex of embodiment 3, wherein the antibody is a Fab fragment.

7. The complex of embodiment 6, wherein the antibody comprises:

(i) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 97; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(ii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 98; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(iii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 99; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(iv) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 100; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 89;

(v) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 100; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 90;

(vi) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 101; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 89;

(vii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 101; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 90;

(viii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 102; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 93;

(ix) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 103; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 95; or

(x) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 102; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 95.

8. The complex of embodiment 6 or embodiment 7, wherein the antibody comprises:

(i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 97; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 98; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(iii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 99; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(iv) a heavy chain comprising the amino acid sequence of SEQ ID NO: 100; and a light chain comprising the amino acid sequence of SEQ ID NO: 89;

(v) a heavy chain comprising the amino acid sequence of SEQ ID NO: 100; and a light chain comprising the amino acid sequence of SEQ ID NO: 90;

(vi) a heavy chain comprising the amino acid sequence of SEQ ID NO: 101; and a light chain comprising the amino acid sequence of SEQ ID NO: 89;

(vii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 101; and a light chain comprising the amino acid sequence of SEQ ID NO: 90;

(viii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 102; and a light chain comprising the amino acid sequence of SEQ ID NO: 93;

(ix) a heavy chain comprising the amino acid sequence of SEQ ID NO: 103; and a light chain comprising the amino acid sequence of SEQ ID NO: 95; or

(x) a heavy chain comprising the amino acid sequence of SEQ ID NO: 102; and a light chain comprising the amino acid sequence of SEQ ID NO: 95.

9. The complex of any one of embodiments 1 to 8, wherein the equilibrium dissociation constant (K_D) of binding of the antibody to the transferrin receptor is in a range from 10^{-11} M to 10^{-6} M.

10. The complex of any one of embodiments 1 to 9, wherein the antibody does not specifically bind to the transferrin binding site of the transferrin receptor and/or wherein the muscle-targeting antibody does not inhibit binding of transferrin to the transferrin receptor.

11. The complex of any one of embodiments 1 to 10, wherein the antibody is cross-reactive with extracellular epitopes of two or more of a human, non-human primate and rodent transferrin receptor.

12. The complex of any one of embodiments 1 to 11, wherein the complex is configured to promote transferrin receptor mediated internalization of the molecular payload into a muscle cell.

13. The complex of any one of embodiments 1 to 12, wherein the antibody is a chimeric antibody, optionally wherein the chimeric antibody is a humanized monoclonal antibody.

14. The complex of any one of embodiments 1 to 13, wherein the antibody is in the form of a ScFv, Fab fragment, Fab' fragment, $F(ab')_2$ fragment, or Fv fragment.

15. The complex of any one of embodiments 1 to 14, wherein the molecular payload is an oligonucleotide.

16. The complex of embodiment 15, wherein the oligonucleotide comprises a sequence listed in Table 14.

16.1. The complex of embodiment 15, wherein the oligonucleotide comprises any one of SEQ ID NO: 437-1241, or is complementary to any one of SEQ ID NO: 1242-2046.

17. The complex of embodiment 16 or embodiment 16.1, wherein the oligonucleotide comprises a region of complementarity to a mutated DMD allele.

18. The complex of any one of embodiments 1 to 14, wherein the molecular payload is a polypeptide.

19. The complex of embodiment 18, wherein the polypeptide is a functional fragment of dystrophin protein.

20. The complex of any one of embodiments 15 to 17, wherein the oligonucleotide is configured to suppress a truncating mutation in a DMD allele by mono- or multi-exon skipping.

21. The complex of any one of embodiments 15 to 17, wherein the oligonucleotide promotes antisense-mediated exon skipping to produce in-frame dystrophin mRNA.

22. The complex of embodiment 21, wherein the oligonucleotide promotes skipping of an exon of DMD in the range of exon 8 to exon 55, optionally exon 23 to exon 53.

23. The complex of embodiment 22, wherein the oligonucleotide promotes skipping of exon 8, exon 23, exon 44, exon 45, exon 50, exon 51, exon 52, exon 53, and/or exon 55.

24. The complex of embodiment 21, wherein the oligonucleotide promotes skipping of exon 51.

25. The complex of embodiment 24, wherein the oligonucleotide promotes skipping of multiple exons in the range of exon 44 to exon 53.

26. The complex of any one of embodiments 15 to 17 or 20 to 25, wherein the oligonucleotide comprises at least one modified internucleotide linkage.

27. The complex of embodiment 26, wherein the at least one modified internucleotide linkage is a phosphorothioate linkage.

28. The complex of embodiment 27, wherein the oligonucleotide comprises phosphorothioate linkages in the Rp stereochemical conformation and/or in the Sp stereochemical conformation.

29. The complex of embodiment 28, wherein the oligonucleotide comprises phosphorothioate linkages that are all in the Rp stereochemical conformation or that are all in the Sp stereochemical conformation.

30. The complex of any one of embodiments 15 to 17 or 20 to 29, wherein the oligonucleotide comprises one or more modified nucleotides.

31. The complex of embodiment 30, wherein the one or more modified nucleotides are 2'-modified nucleotides.

32. The complex of any one of embodiments 15 to 17 or 20 to 31, wherein the oligonucleotide is a gapmer oligonucleotide that directs RNAse H-mediated cleavage of an miRNA that negatively regulates DMD expression in a cell, optionally wherein the miRNA is miR-31.

33. The complex of embodiment 32, wherein the gapmer oligonucleotide comprises a central portion of 5 to 15 deoxyribonucleotides flanked by wings of 2 to 8 modified nucleotides.

34. The complex of embodiment 33, wherein the modified nucleotides of the wings are 2'-modified nucleotides.

35. The complex of any one of embodiments 15 to 17 or 20 to 31, wherein the oligonucleotide is a mixmer oligonucleotide.

36. The complex of embodiment 35, wherein the mixmer oligonucleotide promotes exon skipping.

37. The complex of embodiment 35 or 36, wherein the mixmer oligonucleotide comprises two or more different 2' modified nucleotides.

38. The complex of any one of embodiments 15 to 17 or 20 to 31, wherein the oligonucleotide is an RNAi oligonucleotide that promotes RNAi-mediated cleavage of an miRNA that negatively regulates DMD expression in a cell, optionally wherein the miRNA is miR-31.

39. The complex of embodiment 38, wherein the RNAi oligonucleotide is a double-stranded oligonucleotide of 19 to 25 nucleotides in length.

40. The complex of embodiment 38 or 39, wherein the RNAi oligonucleotide comprises at least one 2' modified nucleotide.

41. The complex of any one of embodiments 31, 34, 37, or 40, wherein each 2' modified nucleotide is selected from the group consisting of: 2′-O-methyl, 2′-fluoro (2′-F), 2′-O-methoxyethyl (2′-MOE), and 2′, 4′-bridged nucleotides.

42. The complex of embodiment 30, wherein the one or more modified nucleotides are bridged nucleotides.

43. The complex of any one of embodiments 31, 34, 37, or 40, wherein at least one 2' modified nucleotide is a 2',4'-bridged nucleotide selected from: 2',4'-constrained 2'-O-ethyl (cEt) and locked nucleic acid (LNA) nucleotides.

44. The complex of any one of embodiments 15 to 17 or 20 to 31, wherein the oligonucleotide comprises a guide sequence for a genome editing nuclease.

45. The complex of any one of embodiments 15 to 17 or 20 to 31, wherein the oligonucleotide is phosphorodiamidate morpholino oligomer.

46. The complex of any one of embodiments 1 to 45, wherein the muscle-targeting agent is covalently linked to the molecular payload via a cleavable linker.

47. The complex of embodiment 46, wherein the cleavable linker is selected from: a protease-sensitive linker, pH-sensitive linker, and glutathione-sensitive linker.

48. The complex of embodiment 47, wherein the cleavable linker is a protease-sensitive linker.

49. The complex of embodiment 48, wherein the protease-sensitive linker comprises a sequence cleavable by a lysosomal protease and/or an endosomal protease.

50. The complex of embodiment 48, wherein the protease-sensitive linker comprises a valine-citrulline dipeptide sequence.

51. The complex of embodiment 47, wherein the linker is pH-sensitive linker that is cleaved at a pH in a range of 4 to 6.

52. The complex of any one of embodiments 1 to 45, wherein the muscle-targeting agent is covalently linked to the molecular payload via a non-cleavable linker.

53. The complex of embodiment 52, wherein the non-cleavable linker is an alkane linker.

54. The complex of any one of embodiments 1 to 53, wherein the antibody comprises a non-natural amino acid to which the oligonucleotide is covalently linked.

55. The complex of any one of embodiments 1 to 53, wherein the antibody is covalently linked to the oligonucleotide via conjugation to a lysine residue or a cysteine residue of the antibody.

56. The complex of embodiment 55, wherein the oligonucleotide is conjugated to the cysteine of the antibody via a maleimide-containing linker, optionally wherein the

maleimide-containing linker comprises a maleimidocaproyl or maleimidomethyl cyclohexane-1-carboxylate group.

57. The complex of any one of embodiments 1 to 56, wherein the antibody is a glycosylated antibody that comprises at least one sugar moiety to which the oligonucleotide is covalently linked.

58. The complex of embodiment 57, wherein the sugar moiety is a branched mannose.

59. The complex of embodiment 57 or 58, wherein the antibody is a glycosylated antibody that comprises one to four sugar moieties each of which is covalently linked to a separate oligonucleotide.

60. The complex of embodiment 57, wherein the antibody is a fully-glycosylated antibody.

61. The complex of embodiment 57, wherein the antibody is a partially-glycosylated antibody.

62. The complex of embodiment 61, wherein the partially-glycosylated antibody is produced via chemical or enzymatic means.

63. The complex of embodiment 61, wherein the partially-glycosylated antibody is produced in a cell that is deficient for an enzyme in the N- or O- glycosylation pathway.

64. A method of delivering a molecular payload to a cell expressing transferrin receptor, the method comprising contacting the cell with the complex of any one of embodiments 1 to 63.

65. A method of promoting the expression or activity of a DMD protein in a cell, the method comprising contacting the cell with the complex of any one of embodiments 1 to 63 in an amount effective for promoting internalization of the molecular payload to the cell.

66. The method of embodiment 65, wherein the cell is *in vitro*.

67. The method of embodiment 65, wherein the cell is in a subject.

68. The method of embodiment 67, wherein the subject is a human.

69. A method of treating a subject having a mutated DMD allele that is associated with a dystrophinopathy, the method comprising administering to the subject an effective amount of the complex of any one of embodiments 1 to 63.

70. A method of promoting skipping of an exon of a DMD mRNA transcript in a cell, the method comprising administering to the cell an effective amount of the complex of any one of embodiments 1 to 63.

71. The method of embodiment 70, wherein the method promotes skipping of exon 8, exon 23, exon 44, exon 45, exon 50, exon 51, exon 52, exon 53, and/or exon 55 of the DMD mRNA transcript.

72. The method of embodiment 70 or 71, wherein the method promotes skipping of exon 51 of the DMD mRNA transcript.

73. A complex comprising an anti-transferrin receptor (TfR) antibody covalently linked to a molecular payload configured for promoting the expression or activity of a DMD gene, wherein the antibody comprises:

(i) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 76; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 75;

(ii) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 69; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 70;

(iii) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 71; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 70;

(iv) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 72; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 70;

(v) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 73; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 74;

(vi) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 73; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 75;

(vii) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 76; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 74;

(viii) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 77; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 78;

(ix) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 79; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 80; or

(x) a heavy chain variable region (VH) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 77; and/or a light chain variable region (VL) comprising an amino acid sequence at least 85% identical to SEQ ID NO: 80.

73. A complex comprising an anti-transferrin receptor (TfR) antibody covalently linked to a molecular payload configured for promoting the expression or activity of a DMD gene, wherein the anti-TfR antibody has undergone pyroglutamate formation resulting from a post-translational modification.

EQUIVALENTS AND TERMINOLOGY

[000610] The disclosure illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the disclosure. Thus, it should be understood that although the present disclosure has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this disclosure.

[000611] In addition, where features or aspects of the disclosure are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

[000612] It should be appreciated that, in some embodiments, sequences presented in the sequence listing may be referred to in describing the structure of an oligonucleotide or other nucleic acid. In such embodiments, the actual oligonucleotide or other nucleic acid may have

one or more alternative nucleotides (e.g., an RNA counterpart of a DNA nucleotide or a DNA counterpart of an RNA nucleotide) and/or (e.g., and) one or more modified nucleotides and/or (e.g., and) one or more modified internucleotide linkages and/or (e.g., and) one or more other modification compared with the specified sequence while retaining essentially same or similar complementary properties as the specified sequence.

[000613] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (*i.e.*, meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.*, "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[000614] Embodiments of this invention are described herein. Variations of those embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description.

The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

What is claimed is:

1. A complex comprising an anti-transferrin receptor (TfR) antibody covalently linked to a molecular payload configured for promoting the expression or activity of a DMD gene, wherein the antibody comprises:

(i) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 76; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 75;

(ii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 69; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 70;

(iii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 71; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 70;

(iv) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 72; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 70;

(v) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 73; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 74;

(vi) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 73; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 75;

(vii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 76; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 74;

(viii) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 77; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 78;

(ix) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 79; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 80; or

(x) a heavy chain variable region (VH) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 77; and/or a light chain variable region (VL) comprising an amino acid sequence at least 95% identical to SEQ ID NO: 80.

2. The complex of claim 1, wherein the antibody comprises:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 76 and a VL comprising the amino acid sequence of SEQ ID NO: 75;

(ii) a VH comprising the amino acid sequence of SEQ ID NO: 69 and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(iii) a VH comprising the amino acid sequence of SEQ ID NO: 71and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(iv) a VH comprising the amino acid sequence of SEQ ID NO: 72 and a VL comprising the amino acid sequence of SEQ ID NO: 70;

(v) a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of SEQ ID NO: 74;

(vi) a VH comprising the amino acid sequence of SEQ ID NO: 73 and a VL comprising the amino acid sequence of SEQ ID NO: 75;

(vii) a VH comprising the amino acid sequence of SEQ ID NO: 76 and a VL comprising the amino acid sequence of SEQ ID NO: 74;

(viii) a VH comprising the amino acid sequence of SEQ ID NO: 77 and a VL comprising the amino acid sequence of SEQ ID NO: 78;

(ix) a VH comprising the amino acid sequence of SEQ ID NO: 79 and a VL comprising the amino acid sequence of SEQ ID NO: 80; or

(x) a VH comprising the amino acid sequence of SEQ ID NO: 77 and a VL comprising the amino acid sequence of SEQ ID NO: 80.

3. The complex of claim 1 or claim 2, wherein the antibody is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab')2 fragment, a scFv, a Fv, and a full-length IgG.

4. The complex of claim 3, wherein the antibody is a Fab fragment.

5. The complex of claim 4, wherein the antibody comprises:

(i) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 101; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 90;

(ii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 97; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(iii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 98; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(iv) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 99; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 85;

(v) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 100; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 89;

(vi) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 100; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 90;

(vii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 101; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 89;

(viii) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 102; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 93;

(ix) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 103; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 95; or

(x) a heavy chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 102; and/or a light chain comprising an amino acid sequence at least 85% identical to SEQ ID NO: 95.

6. The complex of claim 4 or claim 5, wherein the antibody comprises:

(i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 101; and a light chain comprising the amino acid sequence of SEQ ID NO: 90;

(ii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 97; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(iii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 98; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(iv) a heavy chain comprising the amino acid sequence of SEQ ID NO: 99; and a light chain comprising the amino acid sequence of SEQ ID NO: 85;

(v) a heavy chain comprising the amino acid sequence of SEQ ID NO: 100; and a light chain comprising the amino acid sequence of SEQ ID NO: 89;

(vi) a heavy chain comprising the amino acid sequence of SEQ ID NO: 100; and a light chain comprising the amino acid sequence of SEQ ID NO: 90;

(vii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 101; and a light chain comprising the amino acid sequence of SEQ ID NO: 89;

(viii) a heavy chain comprising the amino acid sequence of SEQ ID NO: 102; and a light chain comprising the amino acid sequence of SEQ ID NO: 93;

(ix) a heavy chain comprising the amino acid sequence of SEQ ID NO: 103; and a light chain comprising the amino acid sequence of SEQ ID NO: 95; or

(x) a heavy chain comprising the amino acid sequence of SEQ ID NO: 102; and a light chain comprising the amino acid sequence of SEQ ID NO: 95.

7. The complex of any one of claims 1 to 6, wherein the antibody does not specifically bind to the transferrin binding site of the transferrin receptor and/or wherein the muscle-targeting antibody does not inhibit binding of transferrin to the transferrin receptor.

8. The complex of any one of claims 1 to 7, wherein the molecular payload is an oligonucleotide.

9. The complex of claim 8, wherein the oligonucleotide promotes exon skipping in a DMD RNA.

10. The complex of claim 9, wherein the oligonucleotide promotes skipping of an exon of DMD in the range of exon 8 to exon 55.

11. The complex of claim 9 or claim 10, wherein the oligonucleotide promotes skipping of exon 8, exon 23, exon 43, exon 44, exon 45, exon 46, exon 50, exon 51, exon 52, exon 53, and/or exon 55.

12. The complex of any one of claims 8 to 11, wherein the oligonucleotide comprises a region of complementarity to one or more full or partial exonic splicing enhancers (ESE) of a DMD transcript.

13. The complex of any one of claims 8 to 12, wherein the oligonucleotide comprises a region of complementarity to a target sequence comprising one or more full or partial ESEs as set forth in SEQ ID NOs: 402-436 and 2043-2238.

14. The complex of any one of claims 8 to 13, wherein the oligonucleotide promotes skipping of exon 51.

15. The complex of any one of claims 8 to 14, wherein the oligonucleotide is 20-30 nucleotides in length and comprises a region of complementarity to a target sequence comprising at least 4 consecutive nucleotides of an ESE as set forth in any one of SEQ ID NOs: 402-436.

16. The complex of any one of claims 8 to 14, wherein the oligonucleotide comprises any one of SEQ ID NOs: 437-1241, or comprises a region of complementarity to any one of SEQ ID NOs: 1242-2046.

17. The complex of any one of claims 8-10, wherein the oligonucleotide comprises a region of complementarity to a target sequence of an oligonucleotide listed in Table 14.

18. The complex of any one of claims 8-10 and 17, wherein the oligonucleotide comprises a sequence listed in Table 14, wherein any one or more of the uracil bases (U's) in the oligonucleotide may optionally be a thymine base (T).

19. The complex of any one of claims 8 to 18, wherein the oligonucleotide comprises at least one modified internucleoside linkage.

20. The complex of claim 19, wherein the at least one modified internucleoside linkage is a phosphorothioate linkage.

21. The complex of any one of claims 8 to 20, wherein the oligonucleotide comprises one or more modified nucleosides.

22. The complex of claim 21, wherein the one or more modified nucleosides are 2'-modified nucleosides.

23. The complex of any one of claims 8 to 18, wherein the oligonucleotide comprises one or more phosphorodiamidate morpholinos, optionally wherein the oligonucleotide is a phosphorodiamidate morpholino oligomer (PMO).

24. The complex of any one of claims 1 to 23, wherein the antibody is covalently linked to the molecular payload via a cleavable linker.

25. The complex of claim 24, wherein the cleavable linker comprises a valine-citrulline sequence.

26. The complex of any one of claims 1 to 25, wherein the antibody is covalently linked to the molecular payload via conjugation to a lysine residue or a cysteine residue of the antibody.

27. A method of promoting the expression or activity of a DMD protein in a cell, the method comprising contacting the cell with the complex of any one of claims 1 to 26 in an amount effective for promoting internalization of the molecular payload to the cell, optionally wherein the cell is a muscle cell.

28. A method of treating a subject having a mutated DMD allele that is associated with a dystrophinopathy, the method comprising administering to the subject an effective amount of the complex of any one of claims 1 to 26.