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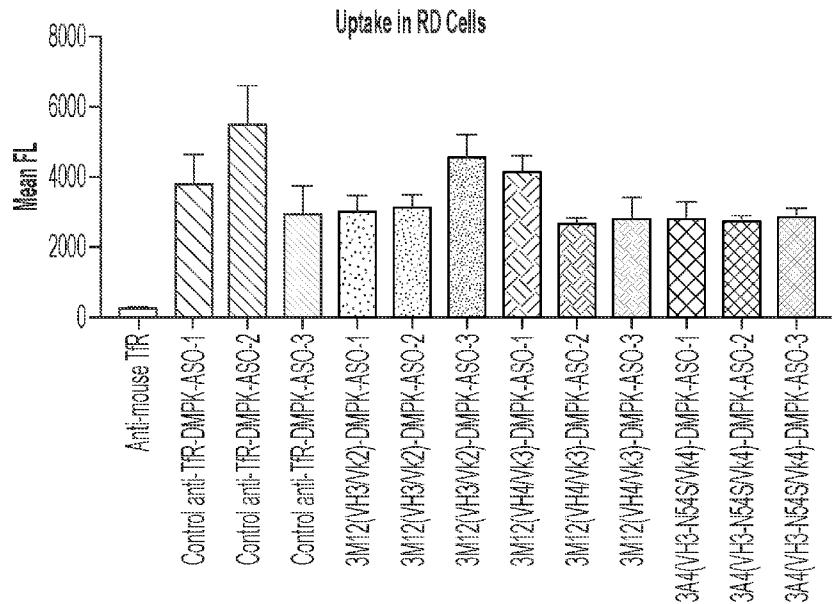


FIG. 2

(57) Abstract: Aspects of the disclosure relate to antibodies that bind to transferrin receptor (e.g., transferrin receptor 1) and complexes comprising the antibody covalently linked to a molecular payload. Methods of using the antibodies are also provided.



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ANTI-TRANSFERRIN RECEPTOR (TFR) ANTIBODY AND USES THEREOF**RELATED APPLICATIONS**

[0001] This application claims priority under 35 U.S.C. § 119(e) to U.S. Provisional Application Serial No. 63/143825, entitled “ANTI-TRANSFERRIN RECEPTOR (TFR) ANTIBODY AND USES THEREOF”, filed on January 30, 2021, to U.S. Provisional Application Serial No. 63/069071, entitled “ANTI-TRANSFERRIN RECEPTOR (TFR) ANTIBODY AND USES THEREOF”, filed on August 23, 2020, and to U.S. Provisional Application Serial No. 63/055721, entitled “ANTI-TRANSFERRIN RECEPTOR (TFR) ANTIBODY AND USES THEREOF”, 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 novel anti-transferrin receptor (TfR) antibodies and the use of the antibodies.

**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 D082470037WO00-SEQ-DWY and is 120,479 bytes in size.

BACKGROUND

[0004] Transferrin Receptor (TfR) is a dimeric transmembrane glycoprotein receptor involved in iron transport. Two transferrin receptors have been characterized in humans, transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2). It has been shown that TfR is overexpressed in cancer cells with higher metastatic potential. TfR1 has been shown to express on the endothelial cells of the blood brain barrier can be used to allow the delivery of large molecules into the brain.

SUMMARY

[0005] The present disclosure is based, at least in part, on the development of humanized antibodies that bind transferrin receptor (anti-TfR antibodies). In some

embodiments, anti-TfR antibodies described herein selectively bind to human or non-human primate (NHP) transferrin receptor 1 (TfR1) with high specificity and affinity (e.g., subnanomolar to nanomolar range). In some embodiments, the anti-TfR antibodies described herein are useful for targeting tissues and/or (e.g., and) cells that express TfR1. In some embodiments, the anti-TfR antibodies provided herein are used for detection of TfR1 in a cell or a tissue. In some embodiments, the anti-TfR antibodies provided herein are used in diagnostic, therapeutic, or research applications. In some embodiments, the anti-TfR antibodies described herein are used to deliver a molecular payload to a target cell or tissue (e.g., a cell or tissue that expresses TfR1).

[0006] As such, in some aspects, complexes comprising the anti-TfR antibodies conjugated (e.g., covalently conjugated) to a molecular payload (e.g., a diagnostic agent or a therapeutic agent) are provided. In some embodiments, the anti-TfR antibodies is used to deliver the conjugated molecular payload to a cell or a tissue that expresses TfR1 (e.g., muscle or the brain) for diagnosing and/or (e.g., and) treating a disease (e.g., a muscle disease or a neurological disease). In some aspects, the present disclosure provides data demonstrating that the anti-TfR antibodies described herein has superior activity in delivering molecular payload into a target cell (e.g., a muscle cell), compared with other known anti-TfR antibodies.

[0007] One aspect of the present disclosure relates to an antibody that binds to human transferrin receptor (TfR), 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.

[0008] 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 an amino acid sequence of SEQ ID NO: 69 and a VL comprising an amino acid sequence of SEQ ID NO: 70;

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

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

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

(vi) a VH comprising an amino acid sequence of SEQ ID NO: 73 and a VL comprising an amino acid sequence of SEQ ID NO: 75;

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

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

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

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

[0009] In some embodiments, the antibody is selected from the group consisting of a Fab fragment, a Fab' fragment, a F(ab')2 fragment, an scFv, an Fv, and a full-length IgG. In some embodiments, the antibody is a Fab fragment.

[00010] 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.

[00011] 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.

[00012] In some embodiments, 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. In some embodiments, the antibody does not specifically bind to the transferrin binding site of the transferrin receptor and/or the antibody does not inhibit binding of transferrin to the transferrin receptor. In some embodiments, the antibody is cross-reactive with extracellular epitopes of two or more of a human, non-human primate and rodent transferrin receptor.

[00013] Another aspect of the present disclosure relates to a complex comprising the antibody covalently linked to a molecular payload. In some embodiments, the molecular payload is a diagnostic agent or a therapeutic agent. In some embodiments, the molecular payload is an oligonucleotide, a polypeptide, or a small molecule. In some embodiments, the

antibody and the molecular payload are linked via a linker. In some embodiments, the linker is a cleavable linker. In some embodiments, the linker comprises a valine-citrulline sequence.

[00014] Another aspect of the present disclosure relates to a composition comprising an antibody or a complex disclosed herein. In some embodiments, the composition further comprises a pharmaceutically acceptable carrier.

[00015] Yet another aspect of the present disclosure relates to a method of delivering a molecular payload to a cell, comprising contacting the cell with a complex or composition disclosed herein. In some embodiments, the cell is a muscle cell. In some embodiments, the cell is in vitro. In some embodiments, the cell is in a subject. In some embodiments, the subject is human.

[00016] Another aspect of the present disclosure relates to a method of delivering a molecular payload to the muscle of a subject, comprising administering to the subject an effective amount of a complex disclosed herein. In some embodiments, the administration is intravenous.

[00017] Another aspect of the present disclosure relates to a method of treating a disease, comprising administering to a subject an effective amount of a complex or a composition disclosed herein, wherein the molecular payload is a therapeutic agent. In some embodiments, the disease is a muscle disease and the molecular payload is a drug for treating the muscle disease. In some embodiments, the muscle disease is a rare muscle disease or muscle atrophy.

BRIEF DESCRIPTION OF THE DRAWINGS

[00018] **FIGs. 1A-1F** show binding of humanized anti-TfR Fabs to human TfR1 (hTfR1) or cynomolgus monkey TfR1 (cTfR1), as measured by ELISA. FIG. 1A shows binding of humanized 3M12 variants to hTfR1. FIG. 1B shows binding of humanized 3M12 variants to cTfR1. FIG. 1C shows binding of humanized 3A4 variants to hTfR1. FIG. 1D shows binding of humanized 3A4 variants to cTfR1. FIG. 1E shows binding of humanized 5H12 variants to hTfR1. FIG. 1F shows binding of humanized 5H12 variants to hTfR1.

[00019] **FIG. 2** 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.

[00020] **FIGs. 3A-3F** 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. 3A shows the binding of humanized 3M12 variants alone or in conjugates with a DMPK targeting oligo to hTfR1. FIG. 3B shows the binding of humanized 3M12 variants alone or in conjugates with a DMPK targeting oligo to cTfR1. FIG. 3C shows the binding of humanized 3A4 variants alone or in conjugates with a DMPK targeting oligo to hTfR1. FIG. 3D shows the binding of humanized 3A4 variants alone or in conjugates with a DMPK targeting oligo to cTfR1. FIG. 3E shows the binding of humanized 5H12 variants alone or in conjugates with a DMPK targeting oligo to hTfR1. FIG. 3F shows the binding of humanized 5H12 variants alone or in conjugates with a DMPK targeting oligo to cTfR1. The respective EC₅₀ values are also shown.

[00021] **FIG. 4** shows DMPK expression in RD cells treated with various concentrations of conjugates containing the indicated humanized anti-TfR Fab antibodies conjugated to a DMPK-targeting oligonucleotide (ASO300). The duration of treatment was 3 days. ASO300 delivered using transfection agents (labeled “Trans”) was used as control.

[00022] **FIG. 5** 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.

[00023] **FIG. 6** 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.

[00024] **FIGs. 7A-7E** show *in vivo* activity of conjugates containing designated anti-TfR Fabs (control, 3M12 VH3/VK2, 3M12 VH4/VK3, and 3A4 VH3 N54S/VK4) conjugated to DMPK targeting oligonucleotide in reducing DMPK mRNA expression in mice expressing human TfR1 (hTfR1 knock-in mice). FIG. 7A shows the experimental design (e.g., IV dosage, dosing frequency). DMPK mRNA levels were measured 14 days post first dose in the tibialis anterior (FIG. 7B), gastrocnemius (FIG. 7C), heart (FIG. 7D), and diaphragm (FIG. 7E), of the mice.

[00025] **FIG. 8** 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.

[00026] **FIG. 9** 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.

[00027] **FIG. 10** 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.

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[00028] The present disclosure, at least in part, is based on the development of humanized anti-TfR antibodies, *e.g.*, antibodies listed in Table 3, which showed high binding affinity and specificity to human TfR. Also provided are the use of the anti-TfR antibodies and their variants in research, diagnostic/detection, and therapeutic applications. In some embodiments, the anti-TfR antibodies described herein are used for delivering molecular payloads (*e.g.*, oligonucleotides, peptides, small molecules) to a target cell or tissue that expresses TfR. In some embodiments, the molecular payload to be delivered is conjugated to the anti-TfR antibodies and delivered to a target cell or tissue that expresses TfR via receptor internalization. Exemplary tissues that express TfR and can be targeted using the anti-TfR antibodies described herein include, without limitation: brain, muscle, adrenal, appendix, bone marrow, colon, duodenum, endometrium, esophagus, fat, gall bladder, heart, kidney, liver, lung, lymph node, ovary, pancreas, placenta, prostate, salivary gland, skin, small intestine, spleen, stomach, testis, thyroid, urinary bladder. In some embodiments, such approach has beneficial effects in muscle cells and for delivering across the blood brain barrier, which have been proven challenging. In some aspects, the present disclosure provides data demonstrating that the anti-TfR antibodies described herein has superior activity in delivering molecular payload into a target cell (*e.g.*, a muscle cell), compared with other known anti-TfR antibodies.

[00029] As such, the present disclosure also provides complexes comprising any one of the anti-TfR1 antibodies covalently linked to molecular payloads. In some embodiments, the complexes are particularly useful for delivering molecular payloads that inhibit the expression

or activity of target genes in muscle cells, e.g., in a subject having or suspected of having a rare muscle disease or muscle atrophy (e.g., as listed in Table 6). In some embodiments, the complexes are particularly useful for delivering drugs to the brain for treating a neurological disease (e.g., as listed in Table 7).

[00030] Further aspects of the disclosure, including a description of defined terms, are provided below.

I. Definitions

[00031] **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).

[00032] **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).

[00033] **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).

[00034] **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.

[00035] 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

	IMGT¹	Kabat²	Chothia³
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))

[00036] 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.

[00037] 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.

[00038] 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.

[00039] 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.

[00040] 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.

[00041] 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.

[00042] 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.

[00043] 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.

[00044] 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-TfR 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.

[00045] 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.

[00046] 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 an anti-TfR antibody. 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.

[00047] 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, phosphorodiamidite 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.

[00048] 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 Lerrick 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.

[00049] Region of complementarity: As used herein, the term "region of complementarity" refers to a nucleotide sequence, e.g., of an 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.

[00050] 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.

[00051] 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 disease-associated-repeat expansion, e.g., in a DMPK allele.

[00052] 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).

[00053] 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:

MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLA VDEEENADNNTKANVTPK
CSGSICYGTIAVIVFFLIGFMIGYLGYCKGVEPKTECERLAGTESPVREEPGEDFPAARRLYWDD

LKRKLSEKLDSTDFTGTIKLLNENSYVPREAGSQKDENLALYVENQFREFKLSKVWRDQHFVK
 IQVKDSAQNSVIIVDKNGRLVYLVENPGGYVAYSAAATVTGKLVHANFGTKKFEDLYTPVN
 GSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVNAELSFFGHAHLGTGDPYTPGFPDF
 NHTQFPPSRSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWKTDSTCRMVTSESKNVKLT
 VLKEIKILNIFGVVIKGFVEPDHYVVVGAQRDAWGPAAKSGVGTALLKLAQMFSDMVLKD
 FQPSRSIIFASWSAGDFGSVGATEWLEGYLSSLHLKAFTYINLDKAVLGTNSFKVSASPLLYTLI
 EKTMQNVKHPVTQFLYQDSNWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYL
 GTTMDTYKELIERIPELNKVARAAAEVAGQFVIKLTHDVELNLDYERYNSQLLSFVRDLNQYRADI
 KEMGLSLQWLYSARGDFRATSRLTTDFRNAEKDRFVMKKLNDRVMRVEYHFLSPYVSPKE
 SPFRHVFWGSGSHTLPALLENLKLRKQNNGAFNETLFRNQLALATWTIQAANALSGDV
 DNEF (SEQ ID NO: 105).

[00054] 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:

MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNHVEMKLGVD
 CGGNICYGTIAVIFFLIGFMIGYLGYCKGVEPKTECERLAGTESPAREEPEEDFPAAPR
 LYWDD
 LKRKLSEKLDTTDFTSTIKLLNENLYV
 PREAGSQKDENLALYIENQFREFKLSKVWRDQHFVKI
 QVKDSAQNSVIIVDKNGGLVYL
 VENPGGYVAYSAAATVTGKLVHANFGTKKFEDLD
 SPVNG
 SIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIV
 KADLSFFGHAHLGTGDPYTPGFPDF
 HTQFPPSQSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWK
 TDSTCKMVTSENKSVKLT
 VSNV
 LKETKILNIFGVVIKGFVEPDHYVVVGAQRDAWGP
 AA
 KSSVGTALLKLAQMFSDMVLKD
 QPSRSIIFASWSAGDFGSVGATEWLEGYLSSLHLKAFTY
 INLDKAVLGTNSFKVSASPLLYTLI
 KTMQDVKHPVTGRSLYQDSNWASKVEKLTLDNAAFP
 FLAYSGIPAVSFCFCEDTDYPYL
 GTTMDTYKELVERIPELNKVARAAAEVAGQFVIKLTHD
 TELNLDYERYNSQLLFLRDLNQYRADI
 VKEMGLSLQWLYSARGDFRATSRLTTDFRNAEKDR
 FVMKKLNDRVMRVEYFLSPYVSP
 KESPFRHVFWGSGSHTLSALLESLKLRQNN
 SAFNETLFRNQLALATWTIQAANALSGDV
 DIDNEF(SEQ ID NO: 106)

[00055] 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:

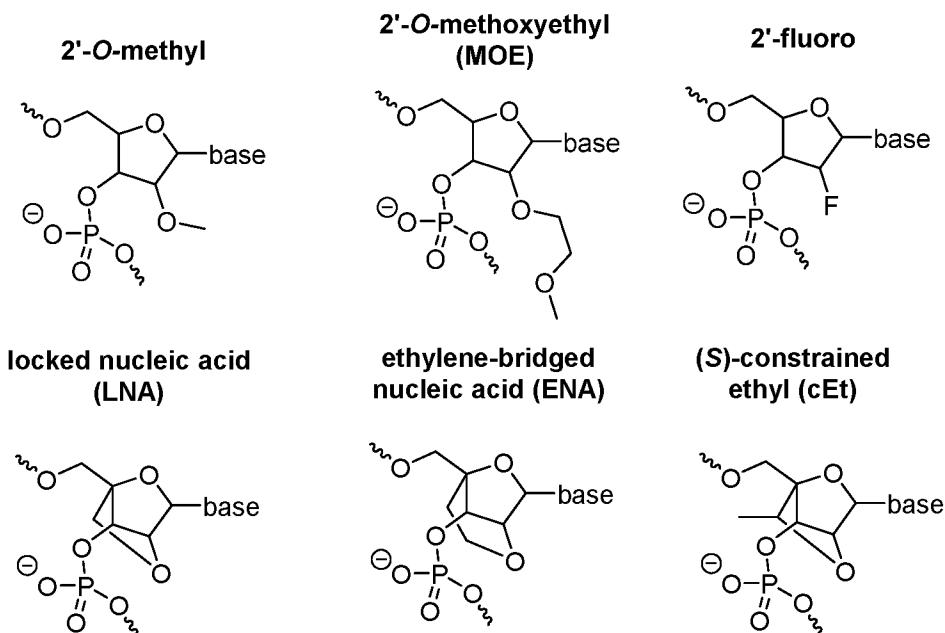
MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNHVEMKLGVD
 CGGNICYGTIAVIFFLIGFMIGYLGYCKGVEPKTECERLAGTESPAREEPEEDFPAAPR
 LYWDD
 LKRKLSEKLDTTDFTSTIKLLNENLYV
 PREAGSQKDENLALYIENQFREFKLSKVWRDQHFVKI
 QVKDSAQNSVIIVDKNGGLVYL
 VENPGGYVAYSAAATVTGKLVHANFGTKKFEDLD
 SPVNG
 SIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIV
 KADLSFFGHAHLGTGDPYTPGFPDF
 HTQFPPSQSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWK
 TDSTCKMVTSENKSVKLT
 VSNV
 LKETKILNIFGVVIKGFVEPDHYVVVGAQRDAWGP
 AA
 KSSVGTALLKLAQMFSDMVLKD
 QPSRSIIFASWSAGDFGSVGATEWLEGYLSSLHLKAFTY
 INLDKAVLGTNSFKVSASPLLYTLI
 KTMQDVKHPVTGRSLYQDSNWASKVEKLTLDNAAFP
 FLAYSGIPAVSFCFCEDTDYPYL
 GTTMDTYKELVERIPELNKVARAAAEVAGQFVIKLTHD
 TELNLDYERYNSQLLFLRDLNQYRADI
 VKEMGLSLQWLYSARGDFRATSRLTTDFRNAEKDR
 FVMKKLNDRVMRVEYFLSPYVSP
 KESPFRHVFWGSGSHTLSALLESLKLRQNN
 SAFNETLFRNQLALATWTIQAANALSGDV
 DIDNEF(SEQ ID NO: 107).

[00056] An example mouse transferrin receptor amino acid sequence, corresponding to NCBI sequence NP_001344227.1 (transferrin receptor protein 1, mus musculus) is as follows:

MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNHVEMKLA
 ADEEENADNNMKASVRKPKR
 FNGRLCFAAIALVIFFLIGFMSGYLG
 YCKRVEQKECVKLAETEETDKSETMETEDVPTSSRLY
 WADLKTLLSEKLN
 SIEFADTIKQLSQNTYTP
 PREAGSQKDES
 LAYYIENQFHEFKFSKV
 WRDEHY
 VKIQVKSSIGQNM
 VTVQSGNLD
 PVE
 SPEGYVAFSKP
 TEVSGKLV
 HANFGTK
 KDFEELSY
 SVN

GSLVIVRAGEITFAEKVANAQSFNAIGVLIYMDKNKFPVVEADLALFGHAHLGTGDPYTPGFP FNHTQFPPSQSSGLPNIPVQTISRAAAEKLFGKMEGSCPARWNIDSSCKLELSQNQNVKLIVKN VLKERRILNIFGVIKGYEEPDRYVVVGAQRDALGAGVAAKSSVGTGLLKLAQVFSDMISKDG FRPSRSIIFASWTAGDFGAVGATELEGYLSSLHLKAFTYINLDKVVLGTSNFKVSASPLLYTL MGKIMQDVKHPVDGKSLYRDSNWKVEKLSFDNAAYPFLAYSGIPAVSFCFCEDADYPYLGT RLDTYEALTQKVPQLNQMVRTAAEVAGQLIILKTHDVELNLDYEMYNKLLSFMKDLNQFKT DIRDMGLSLQWLYSARGDYFRATSLTTDFHNAEKTNRVMREINDRIMKVEYHFLSPYVSPR ESPFRHIFWGSGSHTLSALVENLKLQRQKNITAFNETLFRNQLALATWTIQGVANALSGDIWNID NEF (SEQ ID NO: 108).

[00057] 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-dimethylaminoethoxyethyl (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. Anti-TfR Antibodies

[00058] 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.

[00059] 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.

[00060] In some embodiments, the anti-TfR antibody described herein (e.g., 3M12 and humanized variants) bind an epitope in TfR1, wherein the epitope comprises residues in amino acids 258-291 and/or amino acids 358-381 of SEQ ID NO: 105. In some embodiments, the anti-TfR antibodies (e.g., 3M12 and humanized variants) described herein bind an epitope comprising residues in amino acids amino acids 258-291 and amino acids 358-381 of SEQ ID NO: 105. In some embodiments, the anti-TfR antibodies described herein (e.g., 3M12 and humanized variants) bind an epitope comprising one or more of residues K261, S273, Y282, T362, S368, S370, and K371 of human TfR1 as set forth in SEQ ID NO: 105. In some embodiments, the anti-TfR antibodies described herein (e.g., 3M12 and humanized variants) bind an epitope comprising residues K261, S273, Y282, T362, S368, S370, and K371 of human TfR1 as set forth in SEQ ID NO: 105.

[00061] 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 compete with or inhibit transferrin binding to the TfR1. In some embodiments, binding of any one of the anti-TfR antibody described herein does not compete with or inhibit HFE-beta-2-microglobulin binding to the TfR1.

[00062] 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	EVQLQQSGAELVRPGASVQLSCTASGFNIKDDYMYWVKQRPEQGLEWIGWIDPENGDT EYASKFQDKATVTADTSSNTAYLQLSSLTSEDTAVYYCTLWLRRGLDYWGQGTSVTVS S (SEQ ID NO: 17)		
	VL	DIVMTQAAPSVPVTPGESVSI CRSSKSLLHSNGYTYLFWFLQRPGQSPQLIYRMSNLA 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	EVQLQQSGAELVRPGASV KLSCTASGFNIKDDYMYWVKQRPEQGLEWIGWIDPETGDT EYASKFQDKATVTADTSSNTAYLQLSSLTSEDTAVYYCTLWLRRGLDYWGQGTSVTVS S (SEQ ID NO: 22)		
	VL	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGYTYLFWFLQRPGQSPQLIYRMSNLA SGVPDRFSGSGSGTAFTLISRVEAEDVGVYYCMQHLEYPFTFGGGTKLEIK (SEQ ID NO: 18)		
	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)
3-A4 N54S*	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	EVQLQQSGAELVRPGASV KLSCTASGFNIKDDYMYWVKQRPEQGLEWIGWIDPESGDT EYASKFQDKATVTADTSSNTAYLQLSSLTSEDTAVYYCTLWLRRGLDYWGQGTSVTVS S (SEQ ID NO: 26)		
	VL	DIVMTQAAPSVPVTPGESVSISCRSSKSLLHSNGYTYLFWFLQRPGQSPQLIYRMSNLA SGVPDRFSGSGSGTAFTLISRVEAEDVGVYYCMQHLEYPFTFGGGTKLEIK (SEQ ID NO: 18)		
	CDR-H1	GYSITSGYY (SEQ ID NO: 27)	SGYYWN (SEQ ID NO: 33)	GYSITSGY (SEQ ID NO: 38)
3-M12	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	DVQLQESGPGLVKPSQSLSLTCSVTGYSITSGYYWNWIRQFPGNKLEWMGYITFDGAN NYNPSLKNRISITRDTSKNQFFLKLTSVTTEDTATYYCTRSSYDYDVLDYWGQGTTLTV SS (SEQ ID NO: 43)		
	VL	DIQMTQTSSLASLGDRVTISCRASQDISNFLNWYQQRPDGTVKLLIY YTSRLHSGVPS RFSGSGSGTDFSLTVSNLEQEDIATYFCQQGHTLPYTFGGGTKLEIK (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	QIQLQQSGPELVRPGASVKISCKASGYSFTDYCINWVNQRPGQGLEWIGWIYPGSGNTR YSERFKGKATLTVDTSSNTAYMQLSSLTSEDSAVYFCAREDYYPYHGMDYWGQGTSV TVSS (SEQ ID NO: 61)		
	VL	DIVLTQSPTSLAVSLGQRATISCRASESVDGYDNSFMHWYQQKPGQPPKLLIFRASNLES GIPARFSGSGSRTDFTLTINPVEAADVATYYCQQSSEDPWTFGGGTKEIK (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 GIPARFSGSGSRTDFTLTINPVEAADVATYYCQQSSEDPWTFGGGTKEIK (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 SERFKGKATLTVDTSSNTAYMQLSSLTSEDSAVYFCAREDYYPYHGMDYWGQGTSVT SS (SEQ ID NO: 68)		
	VL	DIVLTQSPTSLAVSLGQRATISCRASESVDGYDNSFMHWYQQKPGQPPKLLIFRASNLES GIPARFSGSGSRTDFTLTINPVEAADVATYYCQQSSEDPWTFGGGTKEIK (SEQ ID NO: 62)		

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

[00063] 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.

[00064] 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.

[00065] 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.

[00066] 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.

[00067] 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).

[00068] 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).

[00069] 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.

[00070] 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.

[00071] 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.

[00072] 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.

[00073] 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.

[00074] 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.

[00075] 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.

[00076] 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.

[00077] 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.

[00078] 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.

[00079] 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.

[00080] 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.

[00081] 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.

[00082] 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.

[00083] 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.

[00084] 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.

[00085] 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.

[00086] 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.

[00087] 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*)/V κ 4	<p>V_H: EVQLVQSGSELKKPGASVKVSCTASGFNIKDDYMYWVRQPPGKGLEWIGWID PETGDTYEASKFQDRVTVTADTSTNTAYMELSSLRSEDTAVYYCTLWLRRGL DYWGQGTLTVSS (SEQ ID NO: 69)</p> <p>V_L: DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGYTYLFWFQQRPGQSPRLLIYR MSNLASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGGGT KVEIK (SEQ ID NO: 70)</p>
3A4 VH3 (N54S*)/V κ 4	<p>V_H: EVQLVQSGSELKKPGASVKVSCTASGFNIKDDYMYWVRQPPGKGLEWIGWID PESGDTYEASKFQDRVTVTADTSTNTAYMELSSLRSEDTAVYYCTLWLRRGL DYWGQGTLTVSS (SEQ ID NO: 71)</p> <p>V_L: DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGYTYLFWFQQRPGQSPRLLIYR MSNLASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGGGT KVEIK (SEQ ID NO: 70)</p>
3A4 VH3 /V κ 4	<p>V_H: EVQLVQSGSELKKPGASVKVSCTASGFNIKDDYMYWVRQPPGKGLEWIGWID PENGDTYEASKFQDRVTVTADTSTNTAYMELSSLRSEDTAVYYCTLWLRRGL DYWGQGTLTVSS (SEQ ID NO: 72)</p> <p>V_L: DIVMTQSPLSLPVTPGEPASISCRSSKSLLHSNGYTYLFWFQQRPGQSPRLLIYR MSNLASGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGGGT KVEIK (SEQ ID NO: 70)</p>
3M12 VH3/V κ 2	<p>V_H: QVQLQESGPGLVKPSQTLSLTCVTGYSITSGYYWNWIRQPPGKGLEWMGYIT FDGANNYNPSLKNRVSISRDTSKNQFSLKLSSVTAEDTATYYCTRSSYDYDVL DYWGQGTTVTVSS (SEQ ID NO: 73)</p> <p>V_L: DIQMTQSPSSLSASVGDRVTITCRASQDISNFLNWYQQKPGQPVKLLIYYTSRL HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQGHTLPYTFGQGKLEIK (SEQ ID NO: 74)</p>
3M12 VH3/V κ 3	<p>V_H: QVQLQESGPGLVKPSQTLSLTCVTGYSITSGYYWNWIRQPPGKGLEWMGYIT FDGANNYNPSLKNRVSISRDTSKNQFSLKLSSVTAEDTATYYCTRSSYDYDVL DYWGQGTTVTVSS (SEQ ID NO: 73)</p> <p>V_L: DIQMTQSPSSLSASVGDRVTITCRASQDISNFLNWYQQKPGQPVKLLIYYTSRL HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGHTLPYTFGQGKLEIK (SEQ ID NO: 75)</p>
3M12 VH4/V κ 2	<p>V_H: QVQLQESGPGLVKPSQTLSLCTVTGYSITSGYYWNWIRQPPGKGLEWIGYITF DGANNYNPSLKNRVSISRDTSKNQFSLKLSSVTAEDTATYYCTRSSYDYDVL DYWGQGTTVTVSS (SEQ ID NO: 76)</p> <p>V_L: DIQMTQSPSSLSASVGDRVTITCRASQDISNFLNWYQQKPGQPVKLLIYYTSRL HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGHTLPYTFGQGKLEIK (SEQ ID NO: 74)</p>
3M12 VH4/V κ 3	<p>V_H: QVQLQESGPGLVKPSQTLSLCTVTGYSITSGYYWNWIRQPPGKGLEWIGYITF DGANNYNPSLKNRVSISRDTSKNQFSLKLSSVTAEDTATYYCTRSSYDYDVL DYWGQGTTVTVSS (SEQ ID NO: 76)</p> <p>V_L: DIQMTQSPSSLSASVGDRVTITCRASQDISNFLNWYQQKPGQPVKLLIYYTSRL HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGHTLPYTFGQGKLEIK (SEQ ID NO: 75)</p>

Antibody	Variable Region Amino Acid Sequence**
5H12 VH5 (C33Y*)/V κ 3	<p>VH: QVQLVQSGAEVKKPGASVKVSCKASGY SFTDYYINWVRQAPGQGLEWMGWI YPGSGNTRYSERFKGRVTITRDT SASTAYMELSSLRSEDTAVYYCAREDYYPY HGMDYWGQGTLTVSS (SEQ ID NO: 77)</p> <p>VL: DIVLTQSPDSLAVSLGERATINCRA SESVDGYDNSFMHWYQQKPGQPPKLLIFR ASNLES GVPDRFSGSGSRTDFTLTISLQAEDVAVYYCQQSSED PWTFGQGK TLEIK (SEQ ID NO: 78)</p>
5H12 VH5 (C33D*)/V κ 4	<p>VH: QVQLVQSGAEVKKPGASVKVSCKASGY SFTDYDINWVRQAPGQGLEWMGWI YPGSGNTRYSERFKGRVTITRDT SASTAYMELSSLRSEDTAVYYCAREDYYPY HGMDYWGQGTLTVSS (SEQ ID NO: 79)</p> <p>VL: DIVMTQSPDSLAVSLGERATINCRA SESVDGYDNSFMHWYQQKPGQPPKLLIF RASNLES GVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQSSED PWTFGQGK TLEIK (SEQ ID NO: 80)</p>
5H12 VH5 (C33Y*)/V κ 4	<p>VH: QVQLVQSGAEVKKPGASVKVSCKASGY SFTDYYINWVRQAPGQGLEWMGWI YPGSGNTRYSERFKGRVTITRDT SASTAYMELSSLRSEDTAVYYCAREDYYPY HGMDYWGQGTLTVSS (SEQ ID NO: 77)</p> <p>VL: DIVMTQSPDSLAVSLGERATINCRA SESVDGYDNSFMHWYQQKPGQPPKLLIF RASNLES GVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQSSED PWTFGQGK TLEIK (SEQ ID NO: 80)</p>

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

** CDRs according to the Kabat numbering system are bolded

[00088] 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.

[00089] 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.

[00090] 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.

[00091] 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.

[00092] 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.

[00093] 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.

[00094] 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.

[00095] 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.

[00096] 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.

[00097] 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.

[00098] 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.

[00099] 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:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPALQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 81)

[000100] 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 Fc γ 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):

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPALQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEA
AGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 82)

[000101] 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)

[000102] 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.

[000103] 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.

[000104] 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.

[000105] 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*)/V κ 4	<p>Heavy Chain (with wild type human IgG1 constant region)</p> <p>EVQLVQSGSELKKPGASVKVSCTASGFNIKDDYMYWVRQPPGKGLEWIGWIDP ETGDTEYASKFQDRVTVTADTSTNTAYMELSSLRSEDTAVYYCTLWLRGLD YWQGQTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNS GALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NYTQKSLSLSPGK (SEQ ID NO: 84)</p> <p>Light Chain (with kappa light chain constant region)</p> <p>DIVMTQSPLSLPVTPGEPASISCRSSKSLHSNGYTYLFWFQORPGQSPRLLIYRM SNLASGVPDFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGGDKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDKDSTYSLSSTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)</p>
3A4 VH3 (N54S*)/V κ 4	<p>Heavy Chain (with wild type human IgG1 constant region)</p> <p>EVQLVQSGSELKKPGASVKVSCTASGFNIKDDYMYWVRQPPGKGLEWIGWIDP ESGDTEYASKFQDRVTVTADTSTNTAYMELSSLRSEDTAVYYCTLWLRGLDY WGQGQTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSG ALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV NKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NYTQKSLSLSPGK (SEQ ID NO: 86)</p> <p>Light Chain (with kappa light chain constant region)</p> <p>DIVMTQSPLSLPVTPGEPASISCRSSKSLHSNGYTYLFWFQORPGQSPRLLIYRM SNLASGVPDFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGGDKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDKDSTYSLSSTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)</p>
3A4 VH3 /V κ 4	<p>Heavy Chain (with wild type human IgG1 constant region)</p> <p>EVQLVQSGSELKKPGASVKVSCTASGFNIKDDYMYWVRQPPGKGLEWIGWIDP ENGDTYEASKFQDRVTVTADTSTNTAYMELSSLRSEDTAVYYCTLWLRGLD YWQGQTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNS GALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NYTQKSLSLSPGK (SEQ ID NO: 87)</p> <p>Light Chain (with kappa light chain constant region)</p> <p>DIVMTQSPLSLPVTPGEPASISCRSSKSLHSNGYTYLFWFQORPGQSPRLLIYRM SNLASGVPDFSGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGGDKVE IKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQ ESVTEQDSKDKDSTYSLSSTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)</p>

Antibody	IgG Heavy Chain/Light Chain Sequences**
3M12 VH3/V κ 2	Heavy Chain (with wild type human IgG1 constant region) QVQLQESGPGLVKPSQLSLTCVTGYSITSGYYWNWIRQPPGKGLEWMGYITF DGANNYNPSLKNRVSISRDTSKNQFSLKLSSVTAEDTATYYCTRSSYDYDVL DY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPV TWSWNSG ALTSGVHTFP AVLQSSGLYSLSSV VTVPSS LTGTQTYICNVN HKPNTKV DKKVE PKSCDKT HCTCPCPAPELLGGPSVFLPPKPKDTLMISRTPE TCVVVDV SHEDPE VKFNWY VDGVEVHN AKTPREEQY NSTYRVV SVLTVL HQDWLNG KEYKCKV NKALPAIEKTISKAKGQPREPQVY TLPPSR DELT KNQVSLTCLVKG FYPSDI AVE WESNGQ PENNYK TTPV LSDGSFFLY SKLT VDKSRW QQGNVF CSV MHEALH NYTQKSLSLSPGK (SEQ ID NO: 88)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC RASQDISNFL NWYQQKPGQPV KLLIYYTSRLH SGVPSRFS SGSGSGTDF LTISLQ PEDFATY FCQQGHTLPY TFGQGT KLEIKRTVA APSVFIFPPSDE QLKSGTASVV CLNNF PREAK VQW KVDN ALQSGNSQESV TEQ DSKDSTY SLSS TL SKADYE HKVY ACEV THQGLSS PVTKSFNR GEC (SEQ ID NO: 89)
3M12 VH3/V κ 3	Heavy Chain (with wild type human IgG1 constant region) QVQLQESGPGLVKPSQLSLTCVTGYSITSGYYWNWIRQPPGKGLEWMGYITF DGANNYNPSLKNRVSISRDTSKNQFSLKLSSVTAEDTATYYCTRSSYDYDVL DY WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPV TWSWNSG ALTSGVHTFP AVLQSSGLYSLSSV VTVPSS LTGTQTYICNVN HKPNTKV DKKVE PKSCDKT HCTCPCPAPELLGGPSVFLPPKPKDTLMISRTPE TCVVVDV SHEDPE VKFNWY VDGVEVHN AKTPREEQY NSTYRVV SVLTVL HQDWLNG KEYKCKV NKALPAIEKTISKAKGQPREPQVY TLPPSR DELT KNQVSLTCLVKG FYPSDI AVE WESNGQ PENNYK TTPV LSDGSFFLY SKLT VDKSRW QQGNVF CSV MHEALH NYTQKSLSLSPGK (SEQ ID NO: 88)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC RASQDISNFL NWYQQKPGQPV KLLIYYTSRLH SGVPSRFS SGSGSGTDF LTISLQ PEDFATY YYCQQGHTLPY TFGQGT KLEIKRTVA APSVFIFPPSDE QLKSGTASVV CLNNF PREAK VQW KVDN ALQSGNSQESV TEQ DSKDSTY SLSS TL SKADYE HKVY ACEV THQGLSS PVTKSFNR GEC (SEQ ID NO: 90)
3M12 VH4/V κ 2	Heavy Chain (with wild type human IgG1 constant region) QVQLQESGPGLVKPSQLSLTCTVTGYSITSGYYWNWIRQPPGKGLEWIGYITFD GANNYNPSLKNRVSISRDTSKNQFSLKLSSVTAEDTATYYCTRSSYDYDVL DY GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPV TWSWNSGA LTSGVHTFP AVLQSSGLYSLSSV VTVPSS LTGTQTYICNVN HKPNTKV DKKVEP KSCDKT HCTCPCPAPELLGGPSVFLPPKPKDTLMISRTPE TCVVVDV SHEDPEV KFNWY VDGVEVHN AKTPREEQY NSTYRVV SVLTVL HQDWLNG KEYKCKVSN KALPAIEKTISKAKGQPREPQVY TLPPSR DELT KNQVSLTCLVKG FYPSDI AVEW ESNGQ PENNYK TTPV LSDGSFFLY SKLT VDKSRW QQGNVF CSV MHEALHNH YTQKSLSLSPGK (SEQ ID NO: 91)
	Light Chain (with kappa light chain constant region) DIQMTQSPSSLSASVGDRVTITC RASQDISNFL NWYQQKPGQPV KLLIYYTSRLH SGVPSRFS SGSGSGTDF LTISLQ PEDFATY FCQQGHTLPY TFGQGT KLEIKRTVA APSVFIFPPSDE QLKSGTASVV CLNNF PREAK VQW KVDN ALQSGNSQESV TEQ DSKDSTY SLSS TL SKADYE HKVY ACEV THQGLSS PVTKSFNR GEC (SEQ ID NO: 89)
3M12 VH4/V κ 3	Heavy Chain (with wild type human IgG1 constant region) QVQLQESGPGLVKPSQLSLTCTVTGYSITSGYYWNWIRQPPGKGLEWIGYITFD GANNYNPSLKNRVSISRDT SKNQFSLKLSSVTAEDTATYYCTRSSYDYDVL DY GQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPV TWSWNSGA LTSGVHTFP AVLQSSGLYSLSSV VTVPSS LTGTQTYICNVN HKPNTKV DKKVEP KSCDKT HCTCPCPAPELLGGPSVFLPPKPKDTLMISRTPE TCVVVDV SHEDPEV KFNWY VDGVEVHN AKTPREEQY NSTYRVV SVLTVL HQDWLNG KEYKCKVSN KALPAIEKTISKAKGQPREPQVY TLPPSR DELT KNQVSLTCLVKG FYPSDI AVEW ESNGQ PENNYK TTPV LSDGSFFLY SKLT VDKSRW QQGNVF CSV MHEALHNH YTQKSLSLSPGK (SEQ ID NO: 91)

Antibody	IgG Heavy Chain/Light Chain Sequences**
	Light Chain (with kappa light chain constant region) <u>DIQMTQSPSSLSASVGDRVTITC</u> RASQDISN <u>FLNWYQQKPGQPVKLLIYYTSRLH</u> <u>SGVPSRFS</u> GSG <u>GTDFTLT</u> ISLQ <u>PEDFATYYC</u> QQGHTLPY <u>TFGQGT</u> KLEIK <u>RTV</u> APSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFRGEC (SEQ ID NO: 90)
5H12 VH5 (C33Y*)/V κ 3	Heavy Chain (with wild type human IgG1 constant region) <u>QVQLVQSGAEVKKPGASV</u> KVSCKASG <u>Y</u> SFTDYY <u>INWVRQAPGQGLEWMG</u> WIY <u>PGSGNTRY</u> SERF <u>KGRV</u> <u>TITRDT</u> S <u>ASTAYMEL</u> SSLR <u>SEDTAVYYC</u> AREDYY <u>PYH</u> <u>GMDYWGQGTL</u> TV <u>SSASTK</u> GPSV <u>FPLAPSS</u> K <u>STSGG</u> T <u>AA</u> LGCL <u>V</u> KD <u>F</u> <u>PEPV</u> TV SWNSGALTSGVHTFPVLQSSGLYSLSSVTVPSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTPVLDSDGSFFLYSKLTVDKSRWQQGVFSCSVMH EALHNHYTQKSLSLSPGK (SEQ ID NO: 92)
5H12 VH5 (C33D*)/V κ 4	Light Chain (with kappa light chain constant region) <u>DIVLTQSPDSLAVSLGERAT</u> INCRA <u>SESV</u> DGYD <u>NSFM</u> HIWYQQKPGQPP <u>KLLI</u> FR <u>SN</u> LESGV <u>PDRF</u> SGSG <u>RTDFTL</u> TISSLQ <u>AEDVA</u> VYYC <u>QOSSE</u> DPW <u>TFGQ</u> G <u>TK</u> LEI <u>KRTV</u> AAPSV <u>FIFPPSDE</u> QLK SGTASVV <u>CLNNF</u> Y PREAKV <u>QWKVD</u> NALQSGNSQ <u>ESVTEQ</u> QDSK <u>DSTYSL</u> SSL TLSKADYE HKVY <u>ACEVTHQGLSSP</u> VTKSFR <u>GEC</u> (SEQ ID NO: 93)
5H12 VH5 (C33Y*)/V κ 4	Heavy Chain (with wild type human IgG1 constant region) <u>QVQLVQSGAEVKKPGASV</u> KVSCKASG <u>Y</u> SFTDYY <u>INWVRQAPGQGLEWMG</u> WIY <u>PGSGNTRY</u> SERF <u>KGRV</u> <u>TITRDT</u> S <u>ASTAYMEL</u> SSLR <u>SEDTAVYYC</u> AREDYY <u>PYH</u> <u>GMDYWGQGTL</u> TV <u>SSASTK</u> GPSV <u>FPLAPSS</u> K <u>STSGG</u> T <u>AA</u> LGCL <u>V</u> KD <u>F</u> <u>PEPV</u> TV SWNSGALTSGVHTFPVLQSSGLYSLSSVTVPSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTPVLDSDGSFFLYSKLTVDKSRWQQGVFSCSVMH EALHNHYTQKSLSLSPGK (SEQ ID NO: 94)
5H12 VH5 (C33Y*)/V κ 4	Light Chain (with kappa light chain constant region) <u>DIVMTQSPDSLAVSLGERAT</u> INCRA <u>SESV</u> DGYD <u>NSFM</u> HWYQQKPGQPP <u>KLLI</u> FR <u>ASN</u> LESGV <u>PDRF</u> SGSG <u>RTDFTL</u> TISSLQ <u>AEDVA</u> VYYC <u>QOSSE</u> DPW <u>TFGQ</u> G <u>TK</u> LEI <u>KRTV</u> AAPSV <u>FIFPPSDE</u> QLK SGTASVV <u>CLNNF</u> Y PREAKV <u>QWKVD</u> NALQSGNSQ <u>ESVTEQ</u> QDSK <u>DSTYSL</u> SSL TLSKADYE HKVY <u>ACEVTHQGLSSP</u> VTKSFR <u>GEC</u> (SEQ ID NO: 95)
5H12 VH5 (C33Y*)/V κ 4	Heavy Chain (with wild type human IgG1 constant region) <u>QVQLVQSGAEVKKPGASV</u> KVSCKASG <u>Y</u> SFTDYY <u>INWVRQAPGQGLEWMG</u> WIY <u>PGSGNTRY</u> SERF <u>KGRV</u> <u>TITRDT</u> S <u>ASTAYMEL</u> SSLR <u>SEDTAVYYC</u> AREDYY <u>PYH</u> <u>GMDYWGQGTL</u> TV <u>SSASTK</u> GPSV <u>FPLAPSS</u> K <u>STSGG</u> T <u>AA</u> LGCL <u>V</u> KD <u>F</u> <u>PEPV</u> TV SWNSGALTSGVHTFPVLQSSGLYSLSSVTVPSLGTQTYICNVNHKPSNTKV DKKVEPKSCDKTHTCPPCAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVNAKTPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSDELTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTPVLDSDGSFFLYSKLTVDKSRWQQGVFSCSVMH EALHNHYTQKSLSLSPGK (SEQ ID NO: 92)
	Light Chain (with kappa light chain constant region) <u>DIVMTQSPDSLAVSLGERAT</u> INCRA <u>SESV</u> DGYD <u>NSFM</u> HWYQQKPGQPP <u>KLLI</u> FR <u>ASN</u> LESGV <u>PDRF</u> SGSG <u>RTDFTL</u> TISSLQ <u>AEDVA</u> VYYC <u>QOSSE</u> DPW <u>TFGQ</u> G <u>TK</u> LEI <u>KRTV</u> AAPSV <u>FIFPPSDE</u> QLK SGTASVV <u>CLNNF</u> Y PREAKV <u>QWKVD</u> NALQSGNSQ <u>ESVTEQ</u> QDSK <u>DSTYSL</u> SSL TLSKADYE HKVY <u>ACEVTHQGLSSP</u> VTKSFR <u>GEC</u> (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

[000106] 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.

[000107] 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.

[000108] 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.

[000109] 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.

[000110] 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.

[000111] 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.

[000112] 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.

[000113] 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.

[000114] 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.

[000115] 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.

[000116] 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.

[000117] 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.

[000118] 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:

ASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVWSNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT (SEQ ID NO: 96)

[000119] 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.

[000120] 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.

[000121] 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*)/V κ 4	Heavy Chain (with partial human IgG1 constant region) <u>EVQLVQSGSELKKPGASVKVSCTASGFNIKDDYMYWVRQPPGKGLEWIGWIDP</u> <u>ETGDTEYASKFQDRVTVTADTSTNTAYMELSSLRSEDTAVYYCTLWLRRGLD</u> <u>YWQGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNS</u> GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV EPKSCDKTHT (SEQ ID NO: 97)
3A4 VH3 (N54S*)/V κ 4	Light Chain (with kappa light chain constant region) <u>DIVMTQSPSLPVTPGEPASISCRSSKSLHSNGYTYLFWFQQRPGQSPRLLIYRM</u> <u>SNLASGVPDFRGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGGGTKVE</u> <u>IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ</u> ESVTEQDSKDKDSTYSLSSTLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)
3A4 VH3 (N54S*)/V κ 4	Heavy Chain (with partial human IgG1 constant region) <u>EVQLVQSGSELKKPGASVKVSCTASGFNIKDDYMYWVRQPPGKGLEWIGWIDP</u> <u>ESGDTEYASKFQDRVTVTADTSTNTAYMELSSLRSEDTAVYYCTLWLRRGLDY</u> <u>WGQGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSG</u> ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV PKSCDKTHT (SEQ ID NO: 98)
3A4 VH3 /V κ 4	Light Chain (with kappa light chain constant region) <u>DIVMTQSPSLPVTPGEPASISCRSSKSLHSNGYTYLFWFQQRPGQSPRLLIYRM</u> <u>SNLASGVPDFRGSGSGTDFTLKISRVEAEDVGVYYCMQHLEYPFTFGGGTKVE</u> <u>IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ</u> ESVTEQDSKDKDSTYSLSSTLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 85)
3M12 VH3/V κ 2	Heavy Chain (with partial human IgG1 constant region) <u>QVQLQESGPGLVKPSQLSLTCVTGYSITSGYYWNWIRQPPGKGLEWMGYITF</u> <u>DGANNYNPSLKNRVSISRDTSKNQFSKLSSVTAEDTATYYCTRSSYDYDVLDY</u> <u>WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSG</u> ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV PKSCDKTHT (SEQ ID NO: 100)
3M12 VH3/V κ 2	Light Chain (with kappa light chain constant region) <u>DIQMTQSPSSLSASVGDRVTITCRASQDISNFLNWYQQKPGQPVKLLIYYTSRLH</u> <u>SGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQQGHTLPYTFGQGKLEIKRTVA</u> APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 89)
3M12 VH3/V κ 3	Heavy Chain (with partial human IgG1 constant region) <u>QVQLQESGPGLVKPSQLSLTCVTGYSITSGYYWNWIRQPPGKGLEWMGYITF</u> <u>DGANNYNPSLKNRVSISRDTSKNQFSKLSSVTAEDTATYYCTRSSYDYDVLDY</u> <u>WGQGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSG</u> ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV PKSCDKTHT (SEQ ID NO: 100)
3M12 VH3/V κ 3	Light Chain (with kappa light chain constant region) <u>DIQMTQSPSSLSASVGDRVTITCRASQDISNFLNWYQQKPGQPVKLLIYYTSRLH</u> <u>SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQQGHTLPYTFGQGKLEIKRTVA</u> APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 90)

Antibody	Fab Heavy Chain/Light Chain Sequences**
3M12 VH4/V κ 2	Heavy Chain (with partial human IgG1 constant region) <u>QVQLQESGPGLVKPSQTLSLTCTVTGYSITSGYYWNWIRQPPGKGLEWIGYITFD</u> <u>GANNYNPSLKNRVSISRDTSKNQFLSKLSSVTAEDTATYYCTRSSYDYDVLVDW</u> <u>GQGTTVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSGA</u> LTSGVHTFPALQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHT (SEQ ID NO: 101)
	Light Chain (with kappa light chain constant region) <u>DIQMTQSPSSLSASVGDRVTITCRASQDISNFLNWYQQKPGQPVKLLIYYTSRLH</u> <u>SGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQGHTLPYTFGQGTTKLEIKRTVA</u> APSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWVKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 89)
3M12 VH4/V κ 3	Heavy Chain (with partial human IgG1 constant region) <u>QVQLQESGPGLVKPSQTLSLTCTVTGYSITSGYYWNWIRQPPGKGLEWIGYITFD</u> <u>GANNYNPSLKNRVSISRDTSKNQFLSKLSSVTAEDTATYYCTRSSYDYDVLVDW</u> <u>GQGTTVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSGA</u> LTSGVHTFPALQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHT (SEQ ID NO: 101)
	Light Chain (with kappa light chain constant region) <u>DIQMTQSPSSLSASVGDRVTITCRASQDISNFLNWYQQKPGQPVKLLIYYTSRLH</u> <u>SGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQGHTLPYTFGQGTTKLEIKRTVA</u> APSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWVKVDNALQSGNSQESVTEQ DSKDSTYSLSSTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 90)
5H12 VH5 (C33Y*)/V κ 3	Heavy Chain (with partial human IgG1 constant region) <u>QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGWIY</u> <u>PGSGNTRYSERFKGRVTITRDTASTAYMELSSLRSEDTAVYYCAREDYYPYH</u> <u>GMDYWGQGTLVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTV</u> SWNSGALTSGVHTFPALQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK DKKVEPKSCDKTHT (SEQ ID NO: 102)
	Light Chain (with kappa light chain constant region) <u>DIVLTQSPDSLAVSLGERATINCRASESVDGYDNSFMHWYQQKPGQPPKLLIFRA</u> <u>SNLESGVPDRFSGSGSRDFTLTISSLQAEDEVAVYYCQOSSEDPTFGQGTTKLEI</u> <u>KRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWVKVDNALQSGNSQ</u> ESVTEQDSKDKSTYSLSSTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 93)
5H12 VH5 (C33D*)/V κ 4	Heavy Chain (with partial human IgG1 constant region) <u>QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYDINWVRQAPGQGLEWMGWIY</u> <u>PGSGNTRYSERFKGRVTITRDTASTAYMELSSLRSEDTAVYYCAREDYYPYH</u> <u>GMDYWGQGTLVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTV</u> SWNSGALTSGVHTFPALQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK DKKVEPKSCDKTHT (SEQ ID NO: 103)
	Light Chain (with kappa light chain constant region) <u>DIVMTQSPDSLAVSLGERATINCRASESVDGYDNSFMHWYQQKPGQPPKLLIFR</u> <u>ASNLESGVPDRFSGSGSGTDFTLTISSLQAEDEVAVYYCQOSSEDPTFGQGTTKLE</u> <u>IKRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWVKVDNALQSGNSQ</u> ESVTEQDSKDKSTYSLSSTLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 95)
5H12 VH5 (C33Y*)/V κ 4	Heavy Chain (with partial human IgG1 constant region) <u>QVQLVQSGAEVKKPGASVKVSCKASGYSFTDYYINWVRQAPGQGLEWMGWIY</u> <u>PGSGNTRYSERFKGRVTITRDTASTAYMELSSLRSEDTAVYYCAREDYYPYH</u> <u>GMDYWGQGTLVTVSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTV</u> SWNSGALTSGVHTFPALQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK DKKVEPKSCDKTHT (SEQ ID NO: 102)

Antibody	Fab Heavy Chain/Light Chain Sequences**
	Light Chain (with kappa light chain constant region) <u>DIVMTQSPDSLAVSLGERATINCRASESVDGYDNSFMH</u> WYQQKPGQPPKLLI <u>R</u> ASN LESGVPDRFSGSGSGTDFLTISLQAEDVAVYYC Q QSSED <u>P</u> WTFGQGT <u>K</u> LE <u>I</u> KRTVAAPSVFIFPPSDEQLKSGTASVVCLNNFYPREAKVQWKVVDNALQSGNSQ ESVTEQDSK <u>D</u> STYSL <u>S</u> STL <u>T</u> SKADY <u>E</u> KHKVYACEVTHQGLSSPVT <u>K</u> SFNR <u>G</u> EC (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

[000122] 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.

[000123] 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.

[000124] 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.

[000125] 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.

[000126] 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.

[000127] 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.

[000128] 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.

[000129] 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.

[000130] 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.

[000131] 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.

[000132] 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.

[000133] 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.

[000134] 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 or C-terminus.

[000135] 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.

[000136] 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.

[000137] 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.

[000138] 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*.

[000139] 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.

[000140] 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).

[000141] 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 C1q 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 Fc γ receptor. This approach is described further in International Publication No. WO 00/42072.

[000142] 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.

[000143] 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.

[000144] 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*”.

[000145] 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).

III Preparation of the Anti-TfR Antibodies

[000146] Antibodies capable of binding TfR as described herein can be made by any method known in the art. *See, for example, Harlow and Lane, (1998) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, New York.*

[000147] In some embodiments, antibodies specific to a target antigen (e.g., TfR) can be made by the conventional hybridoma technology. The full-length target antigen or a fragment thereof, optionally coupled to a carrier protein such as KLH, can be used to immunize a host animal for generating antibodies binding to that antigen. The route and schedule of immunization of the host animal are generally in keeping with established and conventional techniques for antibody stimulation and production, as further described herein. General techniques for production of mouse, humanized, and human antibodies are known in the art and are described herein. It is contemplated that any mammalian subject including humans or antibody producing cells therefrom can be manipulated to serve as the basis for production of mammalian, including human hybridoma cell lines. Typically, the host animal is inoculated intraperitoneally, intramuscularly, orally, subcutaneously, intraplantar, and/or (e.g., and) intradermally with an amount of immunogen, including as described herein.

[000148] If desired, an antibody (monoclonal or polyclonal) of interest (e.g., produced by a hybridoma) may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use. In an alternative, the polynucleotide sequence may be used for genetic manipulation to "humanize" the antibody or to improve the affinity (affinity maturation), or other characteristics of the antibody. For example, the constant region may be engineered to more resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. It may be desirable to genetically manipulate the antibody sequence to obtain greater affinity to the target antigen and greater efficacy. It will be apparent to one of skill in the art that one or more polynucleotide changes can be made to the antibody and still maintain its binding specificity to the target antigen.

[000149] In other embodiments, fully human antibodies can be obtained by using

commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are XenomouseTM from Amgen, Inc. (Fremont, CA) and HuMAb-MouseTM and TC MouseTM from Medarex, Inc. (Princeton, NJ) or H2L2 mice from Harbour Antibodies BV (Holland). In another alternative, antibodies may be made recombinantly by phage display or yeast technology. See, for example, U.S. Pat. Nos. 5,565,332; 5,580,717; 5,733,743; and 6,265,150; and Winter et al., (1994) *Annu. Rev. Immunol.* 12:433-455. Alternatively, the phage display technology (McCafferty et al., (1990) *Nature* 348:552-553) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors.

[000150] Antigen-binding fragments 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. Genetically engineered antibodies, such as humanized antibodies, chimeric antibodies, single-chain antibodies, and bi-specific antibodies, can be produced via, e.g., conventional recombinant technology. In one example, DNA encoding a monoclonal antibodies specific to a target antigen can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into one or more expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, human HEK293 cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. See, e.g., PCT Publication No. WO 87/04462. The DNA can then be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison et al., (1984) *Proc. Nat. Acad. Sci.* 81:6851, or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, genetically engineered antibodies, such as “chimeric” or “hybrid” antibodies; can be prepared that have the binding specificity of a target antigen.

[000151] A single-chain antibody can be prepared via recombinant technology by linking

a nucleotide sequence coding for a heavy chain variable region and a nucleotide sequence coding for a light chain variable region. Preferably, a flexible linker is incorporated between the two variable regions.

[000152] Alternatively, techniques described for the production of single chain antibodies (U.S. Patent Nos. 4,946,778 and 4,704,692) can be adapted to produce a phage or yeast scFv library and scFv clones specific to TfR can be identified from the library following routine procedures. Positive clones can be subjected to further screening to identify those that has high TfR binding affinity.

[000153] Antibodies obtained following a method known in the art and described herein can be characterized using methods well known in the art. For example, one method is to identify the epitope to which the antigen binds, or “epitope mapping.” There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, *Using Antibodies, a Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1999. In one example, epitope mapping can be accomplished use H/D-Ex (hydrogen deuterium exchange) coupled with proteolysis and mass spectrometry. In an additional example, epitope mapping can be used to determine the sequence to which an antibody binds. The epitope can be a linear epitope, i.e., contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch (primary structure linear sequence). Peptides of varying lengths (e.g., at least 4-6 amino acids long) can be isolated or synthesized (e.g., recombinantly) and used for binding assays with an antibody. In another example, the epitope to which the antibody binds can be determined in a systematic screening by using overlapping peptides derived from the target antigen sequence and determining binding by the antibody. According to the gene fragment expression assays, the open reading frame encoding the target antigen is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of the antigen with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled antigen fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide

fragments can be tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen binding domain, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or (e.g., and) necessary for epitope binding. Alternatively, competition assays can be performed using other antibodies known to bind to the same antigen to determine whether an antibody binds to the same epitope as the other antibodies. Competition assays are well known to those of skill in the art.

[000154] In some examples, an anti-TfR antibody is prepared by recombinant technology as exemplified below. Nucleic acids encoding the heavy and light chain of an anti-TfR antibody as described herein can be cloned into one expression vector, each nucleotide sequence being in operable linkage to a suitable promoter. In one example, each of the nucleotide sequences encoding the heavy chain and light chain is in operable linkage to a distinct promoter. Alternatively, the nucleotide sequences encoding the heavy chain and the light chain can be in operable linkage with a single promoter, such that both heavy and light chains are expressed from the same promoter. When necessary, an internal ribosomal entry site (IRES) can be inserted between the heavy chain and light chain encoding sequences.

[000155] In some examples, the nucleotide sequences encoding the two chains of the antibody are cloned into two vectors, which can be introduced into the same or different cells. When the two chains are expressed in different cells, each of them can be isolated from the host cells expressing such and the isolated heavy chains and light chains can be mixed and incubated under suitable conditions allowing for the formation of the antibody.

[000156] Generally, a nucleic acid sequence encoding one or all chains of an antibody can be cloned into a suitable expression vector in operable linkage with a suitable promoter using methods known in the art. For example, the nucleotide sequence and vector can be contacted, under suitable conditions, with a restriction enzyme to create complementary ends on each molecule that can pair with each other and be joined together with a ligase. Alternatively, synthetic nucleic acid linkers can be ligated to the termini of a gene. These synthetic linkers contain nucleic acid sequences that correspond to a particular restriction site in the vector. The selection of expression vectors/promoter would depend on the type of host cells for use in producing the antibodies.

[000157] A variety of promoters can be used for expression of the antibodies described herein, including, but not limited to, cytomegalovirus (CMV) intermediate early promoter, a viral LTR such as the Rous sarcoma virus LTR, HIV-LTR, HTLV-1 LTR, the simian virus 40 (SV40) early promoter, *E. coli lac* UV promoter, and the herpes simplex tk virus promoter.

[000158] Regulatable promoters can also be used. Such regulatable promoters include those using the lac repressor from *E. coli* as a transcription modulator to regulate transcription from lac operator bearing mammalian cell promoters [Brown, M. et al., *Cell*, 49:603-612 (1987)], those using the tetracycline repressor (tetR) [Gossen, M., and Bujard, H., *Proc. Natl. Acad. Sci. USA* 89:5547-555115 (1992); Yao, F. et al., *Human Gene Therapy*, 9:1939-1950 (1998); Shockelt, P., et al., *Proc. Natl. Acad. Sci. USA*, 92:6522-6526 (1995)]. Other systems include FK506 dimer, VP16 or p65 using astradiol, RU486, diphenol murislerone, or rapamycin. Inducible systems are available from Invitrogen, Clontech and Ariad, among others.

[000159] Regulatable promoters that include a repressor with the operon can be used. In one embodiment, the lac repressor from *E. coli* can function as a transcriptional modulator to regulate transcription from lac operator-bearing mammalian cell promoters [M. Brown et al., *Cell*, 49:603-612 (1987)]; Gossen and Bujard (1992); [M. Gossen et al., *Natl. Acad. Sci. USA*, 89:5547-5551(1992)] combined the tetracycline repressor (tetR) with the transcription activator (VP 16) to create a tetR-mammalian cell transcription activator fusion protein, tTa (tetR-VP 16), with the tetO bearing minimal promoter derived from the human cytomegalovirus (hCMV) promoter to create a tetR-tet operator system to control gene expression in mammalian cells. In one embodiment, a tetracycline inducible switch is used. The tetracycline repressor (tetR) alone, rather than the tetR-mammalian cell transcription factor fusion derivatives can function as potent trans-modulator to regulate gene expression in mammalian cells when the tetracycline operator is properly positioned downstream for the TATA element of the CMVIE promoter (Yao et al., *Human Gene Therapy*). One particular advantage of this tetracycline inducible switch is that it does not require the use of a tetracycline repressor-mammalian cells transactivator or repressor fusion protein, which in some instances can be toxic to cells (Gossen et al., *Natl. Acad. Sci. USA*, 89:5547-5551 (1992); Shockett et al., *Proc. Natl. Acad. Sci. USA*, 92:6522-6526 (1995)), to achieve its regulatable effects.

[000160] Additionally, the vector can contain, for example, some or all of the following: a selectable marker gene, such as the neomycin gene for selection of stable or transient transfectants in mammalian cells; enhancer/promoter sequences from the immediate early gene of human CMV for high levels of transcription; transcription termination and RNA processing signals from SV40 for mRNA stability; SV40 polyoma origins of replication and ColE1 for proper episomal replication; internal ribosome binding sites (IRESes), versatile multiple cloning sites; and T7 and SP6 RNA promoters for in vitro transcription of sense and antisense

RNA. Suitable vectors and methods for producing vectors containing transgenes are well known and available in the art. Examples of polyadenylation signals useful to practice the methods described herein include, but are not limited to, human collagen I polyadenylation signal, human collagen II polyadenylation signal, and SV40 polyadenylation signal.

[000161] One or more vectors (e.g., expression vectors) comprising nucleic acids encoding any of the antibodies may be introduced into suitable host cells for producing the antibodies. The host cells can be cultured under suitable conditions for expression of the antibody or any polypeptide chain thereof. Such antibodies or polypeptide chains thereof can be recovered by the cultured cells (e.g., from the cells or the culture supernatant) via a conventional method, e.g., affinity purification. If necessary, polypeptide chains of the antibody can be incubated under suitable conditions for a suitable period of time allowing for production of the antibody.

[000162] In some embodiments, methods for preparing an antibody described herein involve a recombinant expression vector that encodes both the heavy chain and the light chain of an anti-TfR antibody, as also described herein. The recombinant expression vector can be introduced into a suitable host cell (e.g., a dhfr- CHO cell) by a conventional method, e.g., calcium phosphate mediated transfection. Positive transformant host cells can be selected and cultured under suitable conditions allowing for the expression of the two polypeptide chains that form the antibody, which can be recovered from the cells or from the culture medium. When necessary, the two chains recovered from the host cells can be incubated under suitable conditions allowing for the formation of the antibody. In some embodiments, the host cell used for expressing the anti-TfR antibodies described herein are CHO-S cells (e.g., ThermoFisher Catalog# R80007).

[000163] In one example, two recombinant expression vectors are provided, one encoding the heavy chain of the anti-TfR antibody and the other encoding the light chain of the anti-TfR antibody. Both of the two recombinant expression vectors can be introduced into a suitable host cell (e.g., dhfr- CHO cell) by a conventional method, e.g., calcium phosphate-mediated transfection.

[000164] Alternatively, each of the expression vectors can be introduced into a suitable host cells. Positive transformants can be selected and cultured under suitable conditions allowing for the expression of the polypeptide chains of the antibody. When the two expression vectors are introduced into the same host cells, the antibody produced therein can be recovered from the host cells or from the culture medium. If necessary, the polypeptide chains can be recovered from the host cells or from the culture medium and then incubated under suitable

conditions allowing for formation of the antibody. When the two expression vectors are introduced into different host cells, each of them can be recovered from the corresponding host cells or from the corresponding culture media. The two polypeptide chains can then be incubated under suitable conditions for formation of the antibody.

[000165] Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recovery of the antibodies from the culture medium. For example, some antibodies can be isolated by affinity chromatography with a Protein A or Protein G coupled matrix.

[000166] 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.

[000167] 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.

IV. Complexes

[000168] In some embodiments, the humanized anti-TfR antibodies described herein can be used for delivering a molecular payload to a target cell or a target tissue (e.g., a cell or tissue that expresses TfR). Accordingly, some aspects of the present disclosure provide complexes comprising any one of the humanized anti-TfR antibody described herein (e.g., humanized 3-A4, 3-M12, or 5-H12 in IgG or Fab form as provided in Table 4 and Table 5) to a molecular payload. The complexes described herein may be used in various applications, e.g., diagnostic or therapeutic applications.

[000169] In some embodiments, a complex comprises an anti-TfR antibody covalently linked to an oligonucleotide (e.g., an antisense oligonucleotide). In some embodiments, the complex described herein is 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.

A. Molecular Payloads

[000170] Some aspects of the disclosure provide molecular payloads, e.g., for modulating a biological outcome, e.g., the transcription of a DNA sequence, the expression of a protein, or the activity of a protein, that can be linked to any one of the anti-TfR antibodies described herein. 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 the linked anti-TfR antibody. It should be appreciated that various types of molecular payloads 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).

[000171] In some embodiments, the molecular payload is an oligonucleotide that comprises a strand having a region of complementarity to a gene provided in Table 6.

Table 6. List of muscle diseases and corresponding genes.

Rare Muscle Disease Target Genes		
Disease	Gene Symbol	GenBank Accession No.
Adult Pompe	GAA	NM_000152; NM_001079803; NM_001079804
Adult Pompe	GYS1	NM_001161587; NM_002103
Centronuclear myopathy (CNM)	DNM2	NM_001190716; NM_004945; NM_001005362; NM_001005360; NM_001005361; NM_007871
Duchenne muscular dystrophy	DMD	NM_004023; NM_004020; NM_004018; NM_004012
Facioscapulohumeral muscular dystrophy (FSHD)	DUX4	NM_001306068; NM_001363820; NM_001205218; NM_001293798
Familial hypertrophic cardiomyopathy	MYBPC3	NM_000256

Familial hypertrophic cardiomyopathy	MYH6	NM_002471; NM_001164171; NM_010856
Familial hypertrophic cardiomyopathy	MYH7	NM_000257; NM_080728
Familial hypertrophic cardiomyopathy	TNNI3	NM_000363
Familial hypertrophic cardiomyopathy	TNNT2	NM_001001432; NM_001001431; NM_000364; NM_001001430; NM_001276347; NM_001276346; NM_001276345
Fibrodysplasia Ossificans Progressiva (FOP)	ACVR1	NM_001105; NM_001347663; NM_001347664; NM_001347665; NM_001347666; NM_001347667; NM_001111067
Friedreich's ataxia (FRDA)	FXN	NM_001161706; NM_181425; NM_000144
Inclusion body myopathy 2	GNE	NM_001190383; NM_001190384; NM_001128227; NM_005476; NM_001190388
Laing distal myopathy	MYH7	NM_000257; NM_080728
Myofibrillar myopathy	BAG3	NM_004281
Myofibrillar myopathy	CRYAB	NM_001885; NM_001330379; NM_001289807; NM_001289808
Myofibrillar myopathy	DES	NM_001927
Myofibrillar myopathy	DNAJB6	NM_005494; NM_058246
Myofibrillar myopathy	FHL1	NM_001159701; NM_001159699; NM_001159702; NM_001159703; NM_001159704; NM_001159700; NM_001167819; NM_001330659; NM_001449; NM_001077362
Myofibrillar myopathy	FLNC	NM_001458; NM_001127487
Myofibrillar myopathy	LDB3	NM_007078; NM_001171611; NM_001171610; NM_001080114; NM_001080115; NM_001080116
Myofibrillar myopathy	MYOT	NM_001300911; NM_006790; NM_001135940
Myofibrillar myopathy	PLEC	NM_201378; NM_201379; NM_201380; NM_201381; NM_201382; NM_201383; NM_201384; NM_000445
Myofibrillar myopathy	TTN	NM_133432; NM_133379; NM_133437; NM_003319;

		NM_001256850; NM_001267550; NM_133378
Myotonia congenita (autosomal dominant form, Thomsen Disease)	CLCN1	NM_000083; NM_013491
Myotonic dystrophy type I	DMPK	NM_001081563; NM_004409; NM_001081560; NM_001081562; NM_001288764; NM_001288765; NM_001288766
Myotonic dystrophy type II	CNBP	NM_001127192; NM_001127193; NM_001127194; NM_001127195; NM_001127196; NM_003418
Myotubular myopathy	MTM1	NM_000252
Oculopharyngeal muscular dystrophy	PABPN1	NM_004643
Paramyotonia congenita	SCN4A	NM_000334

Muscle Atrophy Gene Targets

Gene Symbol	GenBank Accession No.	Related Publications*
INHBA (also known as EDF; FRP)	NM_002192; XM_017012175.1; XM_017012176.1; XM_017012174.1	Lee SJ, et al., Regulation of muscle mass by follistatin and activins., <i>Mol Endocrinol.</i> 2010 Oct;24(10):1998-2008. doi: 10.1210/me.2010-0127. Epub 2010 Sep 1.
FBXO32 (also known as Fbx32; MAFbx)	NM_058229.3; NM_001242463.1; NM_148177.2	Bodine, S. C., et al., Identification of ubiquitin ligases required for skeletal muscle atrophy. <i>Science</i> 294: 1704-1708, 2001. Gomes, M. D., et al., Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. <i>Proc. Nat. Acad. Sci.</i> 98: 14440-14445, 2001.
MSTN (also known as GDF8; MSLHP)	NM_005259.2	Saunders, M. A., et al., Human adaptive evolution of myostatin (GDF8), a regulator of muscle growth. <i>Am. J. Hum. Genet.</i> 79: 1089-1097, 2006. Lin, J., et al., Myostatin knockout in mice increases myogenesis and decreases adipogenesis. <i>Biochem. Biophys. Res. Commun.</i> 291: 701-706, 2002.

		Wei Y, et al., Prevention of Muscle Wasting by CRISPR/Cas9-mediated Disruption of Myostatin In Vivo Volume 24, Issue 11, p1889–1891, November 2016
TRIM63 (also known as IRF; SMRZ; MURF1; MURF2; RNF28)	NM_032588.3; XM_017002559.2	Höllriegel R, et al. Anabolic effects of exercise training in patients with advanced chronic heart failure (NYHA IIIb): impact on ubiquitin-protein ligases expression and skeletal muscle size. <i>Int J Cardiol</i> , 2013 Aug 10. Eddins MJ, et al. Targeting the ubiquitin E3 ligase MuRF1 to inhibit muscle atrophy. <i>Cell Biochem Biophys</i> , 2011 Jun.

*The contents of the cited references are incorporated herein by reference in their entireties.

[000172] In some embodiments, the molecular payload is an agent for the treatment of a neurological disorder. A “neurological disorder” as used herein refers to a disease or disorder which affects the CNS and/or (e.g., and) which has an etiology in the CNS. Examples of CNS diseases or disorders include, but are not limited to, neuropathy, amyloidosis, cancer, an ocular disease or disorder, viral or microbial infection, inflammation, ischemia, neurodegenerative disease, seizure, behavioral disorders, and a lysosomal storage disease. For the purposes of this application, the CNS will be understood to include the eye, which is normally sequestered from the rest of the body by the blood-retina barrier. Specific examples of neurological disorders include, but are not limited to, neurodegenerative diseases (including, but not limited to, Lewy body disease, postpoliomyelitis syndrome, Shy-Draeger syndrome, olivopontocerebellar atrophy, Parkinson's disease, multiple system atrophy, striatonigral degeneration, tauopathies (including, but not limited to, Alzheimer disease and supranuclear palsy), prion diseases (including, but not limited to, bovine spongiform encephalopathy, scrapie, Creutzfeldt-Jakob syndrome, kuru, Gerstmann-Straussler-Scheinker disease, chronic wasting disease, and fatal familial insomnia), bulbar palsy, motor neuron disease, and nervous system heterodegenerative disorders (including, but not limited to, Canavan disease, Huntington's disease, neuronal ceroid-lipofuscinosis, Alexander's disease, Tourette's syndrome, Menkes kinky hair syndrome, Cockayne syndrome, Halervorden-Spatz syndrome, lafora disease, Rett syndrome, hepatolenticular degeneration, Lesch-Nyhan syndrome, and Unverricht-Lundborg syndrome), dementia (including, but not limited to, Pick's disease, and spinocerebellar ataxia), cancer (e.g. of the CNS, including brain metastases resulting from cancer elsewhere in the body). Non-

limiting, examples of neurological disorder drugs that may be conjugated to any one of the anti-TfR antibodies described herein and the corresponding conditions they may treat are provided in Table 7.

Table 7. Examples of neurological disorder drugs and conditions treated

Drug	Neurological disorder
Anti-BACE1 Antibody	Alzheimer's, acute and chronic brain injury, stroke
Anti-Abeta Antibody	Alzheimer's disease
Anti-Tau Antibody	Alzheimer's disease, taupathies
Neurotrophin	Stroke, acute brain injury, spinal cord injury
Brain-derived neurotrophic factor (BDNF), Fibroblast growth factor 2 (FGF-2)	Chronic brain injury (Neurogenesis)
Anti-Epidermal Growth Factor Receptor (EGFR)-antibody	Brain Cancer
Glial cell-line derived neural factor (GDNF)	Parkinson's disease
Brain derived neurotrophic factor (BDNF)	Amyotrophic lateral sclerosis, depression
Lysosomal enzyme	Lysosomal storage disorders of the brain
Ciliary neurotrophic factor (CNTF)	Amyotrophic lateral sclerosis
Neuregulin-1	Schizophrenia
Anti-HER2 antibody (e.g. trastuzumab, pertuzumab, etc.)	Brain metastasis from HER2-positive cancer
Anti-BEGF antibody (e.g. bevacizumab)	Recurrent or newly diagnosed glioblastoma, recurrent malignant glioma, brain metastasis

[000173] In some embodiments, at least one (e.g., at least 2, at least 3, at least 4, at least 5, at least 10) molecular payload (e.g., oligonucleotides) is linked to any one of the anti-TfR antibody described herein. In some embodiments, all molecular payloads attached to the anti-TfR antibody are the same, e.g. target the same gene. In some embodiments, all molecular payloads attached to the anti-TfR antibody are different, for example the molecular payloads may target different portions of the same target gene, or the molecular payloads may target at least two different target genes. In some embodiments, an anti-TfR antibody described herein may be attached to some molecular payloads that are the same and some molecular payloads that are different.

[000174] The present disclosure also provides a composition comprising a plurality of complexes, for which at least 80% (e.g., 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%, or at least 99%) of the complexes comprise an anti-TfR antibody linked to the same number of molecular payloads (e.g., oligonucleotides).

[000175] 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

[000176] Any suitable oligonucleotide may be used as a molecular payload, as described herein. 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, an oligonucleotide may be a guide nucleic acid (e.g., guide RNA) for directing activity of an enzyme (e.g., a gene editing enzyme). 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.

In some embodiments, an oligonucleotide may comprise a region of complementarity to a target gene provided in Table 6.

[000177] 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.

[000178] In some embodiments, any one of the oligonucleotides can be in salt form, e.g., as sodium, potassium, or magnesium salts.

[000179] 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)₂NR^A-, -NR^AS(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.

[000180] In some embodiments, the 5' or 3' nucleoside of any one of the oligonucleotides described herein is conjugated to a compound of the formula -NH₂-(CH₂)_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 NH₂-(CH₂)_n- and the 5' or 3' nucleoside of the oligonucleotide. In some embodiments, a compound of the formula NH₂-(CH₂)₆- is conjugated to the oligonucleotide via a reaction between 6-amino-1-hexanol (NH₂-(CH₂)₆-OH) and the 5' phosphate of the oligonucleotide.

[000181] 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

[000182] 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.

[000183] 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 normal function of the target (e.g., mRNA) to cause a loss of activity (e.g., inhibiting translation) 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.

[000184] 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.

[000185] 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 herein. In some embodiments, such target sequence is 100% complementary to the oligonucleotide provided herein.

[000186] In some embodiments, any one or more of the thymine bases (T's) in any one of the oligonucleotides provided herein may optionally be uracil bases (U's), and/or any one or more of the U's may optionally be T's.

b. Oligonucleotide Modifications:

[000187] 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.

[000188] 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.

[000189] 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

[0001] 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.

[0002] 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-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA) modified nucleoside.

[0003] 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.

[0004] 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.

[0005] 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.

[0006] 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).

[0007] 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).

[0008] 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

[0009] 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.

[00010] 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.

[00011] In some embodiments, oligonucleotides may have heteroatom backbones, such as methylene(methylimino) or MMI backbones; amide backbones (see De Mesmaeker et al. *Acc. 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

[000190] 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

[000191] In some embodiments, the oligonucleotide may be a morpholino-based compound. 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)

[000192] 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

[000193] 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.

[000194] 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.

[000195] 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 internucleoside linkages. 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.

[000196] 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.

[000197] 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.

[000198] 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.

[000199] 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).

[000200] 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.

[000201] 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 nucleoside (e.g., high-affinity modified nucleosides) is a 2'-modified 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-dimethylaminoethoxyethyl (2'-O-DMAEOE), or 2'-O-N-methylacetamido (2'-O-NMA)).

[000202] 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.

[000203] 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).

[000204] 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).

[000205] 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.

[000206] 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).

[000207] 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.

[000208] 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-BBBA; KKK-(D)n-KKAA; LLL-(D)n-LLAA; BBB-(D)n-BBEE; KKK-(D)n-KKKE; LLL-(D)n-LLEE; BBB-(D)n-BBAA; KKK-(D)n-KKAA; LLL-(D)n-LLAA; BBB-(D)n-BBEE; KKK-(D)n-KKKE; LLL-(D)n-LLEE; BBB-(D)n-BBAAA; KKK-(D)n-KKKAAA; LLL-(D)n-LLAAA; BBB-(D)n-BBBEEE; KKK-(D)n-KKKEEE; LLL-(D)n-LLEEE; BBB-(D)n-BBAAA; KKK-(D)n-KKKAAA; LLL-(D)n-LLAAA; BBB-(D)n-BBBEEE; KKK-(D)n-KKKEEE; LLL-(D)n-LLEEE; 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-LLA; EBBB-(D)n-BBEE; EKKK-(D)n-KKKE; ELLL-(D)n-LLLE; ABBB-(D)n-BBBA; AKKK-(D)n-KKKA; ALLL-(D)n-LLA; EBBB-(D)n-BBEE; EKKK-(D)n-KKKE; ELLL-(D)n-LLLE; ABBB-(D)n-BBAA; AKKK-(D)n-KKAA; ALLL-(D)n-LLAA; EBBB-(D)n-BBEE; EKKK-(D)n-KKKE; ELLL-(D)n-LLLE; ABBB-(D)n-BBB; AAKKK-(D)n-KKK; ALLL-(D)n-LLL; EEBBB-(D)n-BBB; EKKK-(D)n-KKK; ELLL-(D)n-LLL; AABBB-(D)n-BBB; AAKKK-(D)n-KKK; ALLL-(D)n-LLL; EEBBB-(D)n-BBB; EKKK-(D)n-KKK; ELLL-(D)n-LLL; AABBB-(D)n-BBBA; AAKKK-(D)n-KKKA; ALLL-(D)n-LLA; EEBBB-(D)n-BBEE; EKKK-(D)n-KKKE; ELLL-(D)n-LLLE; AABBB-(D)n-BBBA; AAKKK-(D)n-KKKA; ALLL-(D)n-LLA; EEBBB-(D)n-BBEE; EKKK-(D)n-KKKE; ELLL-(D)n-LLLE; ABBAABB-(D)n-BB; AKKAACK-(D)n-KK; ALLAALLL-(D)n-LL; EBBEEBB-(D)n-BB; EKKEEKK-(D)n-KK; ELLEELL-(D)n-LL; ABBAABB-(D)n-BB; AKKAACK-(D)n-KK; ALLAALL-(D)n-LL; EBBEEBB-(D)n-BB; EKKEEKK-(D)n-KK; ELLEELL-(D)n-LL; ABBABB-(D)n-BBB; AKKAACK-(D)n-KKK; ALLALL-(D)n-LLL; EBBEBB-(D)n-BBB; EKKEKK-(D)n-KKK; ELLELL-(D)n-LLL; EEEK-(D)n-EEEEEEEEE; EK-(D)n-EEEEEEEEE; EK-(D)n-EEEKK; K-(D)n-EEEKEKE; K-(D)n-EEEKEKE; K-(D)n-EEKEK; EK-(D)n-EEEKEKE; EK-(D)n-EEEKEK; EK-(D)n-KEEKE; EK-(D)n-EEKEK; EK-(D)n-KEEK; EK-(D)n-EEEKEK; EK-(D)n-KEEEKEE; EK-(D)n-EEKEKE; EK-(D)n-EEEKEKE; and EK-(D)n-EEEKEK; “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.

[000209] 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

[000210] 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.

[000211] 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.

[000212] 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.

[000213] 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.

[000214] 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.

[000215] 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.

[000216] 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).

[000217] 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)

[000218] 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.

[000219] 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 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.

[000220] 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.

[000221] 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.

[000222] 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.

[000223] 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.

[000224] 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.

[000225] 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 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.

[000226] 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.

[000227] 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.

[000228] 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-dimethylaminoethoxyethyl (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.

[000229] 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.

[000230] 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.

[000231] 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.

[000232] 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-dimethylaminoethoxyethyl (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).

[000233] 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.

[000234] 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.

[000235] 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-dimethylaminoethoxyethyl (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.

[000236] 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,

thionophosphoramides, 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.

[000237] 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.

[000238] 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.

[000239] 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 acid (UNA) modification, which reduce RISC loading of the sense strand and improve

silencing potency 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 embodiments, the sense strand of the siRNA molecule comprises a 5-nitroindole modification, which decreased the RNAi potency of the sense strand and reduces off-target 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)

[000240] 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.

[000241] 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.

I. Aptamers

[000242] 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

[000243] 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.

[000244] 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.

[000245] 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.

[000246] 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

[000247] 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.

[000248] 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.

[000249] 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

[000250] 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/conjugate can be increased beyond the available linking sites on a targeting agent (e.g., available thiol sites or amine 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).

[000251] 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.

[000252] 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 an 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.

[000253] 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.

o. Splice Altering Oligonucleotides

[000254] In some embodiments, an oligonucleotide (*e.g.*, an antisense oligonucleotide including a morpholino) of the present disclosure target splicing. In some embodiments, the oligonucleotide targets splicing by inducing exon skipping and restoring the reading frame within a gene. As a non-limiting example, the oligonucleotide may induce skipping of an exon encoding a frameshift mutation and/or (*e.g.*, and) an exon that encodes a premature stop codon. In some embodiments, an oligonucleotide may induce exon skipping by blocking spliceosome recognition of a splice site. In some embodiments, exon skipping results in a truncated but functional protein compared to the reference protein (*e.g.*, truncated but functional DMD protein as described below). In some embodiments, the oligonucleotide promotes inclusion of a particular exon (*e.g.*, exon 7 of the SMN2 gene described below). In some embodiments, an oligonucleotide may induce inclusion of an exon by targeting a splice site inhibitory sequence. RNA splicing has been implicated in muscle diseases, including Duchenne muscular dystrophy (DMD) and spinal muscular atrophy (SMA).

[000255] Alterations (*e.g.*, deletions, point mutations, and duplications) in the gene encoding dystrophin (DMD) cause DMD. These alterations can lead to frameshift mutations and/or (*e.g.*, and) nonsense mutations. In some embodiments, an oligonucleotide of the present disclosure promotes skipping of one or more DMD exons (*e.g.*, exon 8, exon 43, exon 44, exon 45, exon 50, exon 51, exon 52, exon 53, and/or (*e.g.*, and) exon 55) and results in a functional truncated protein. See, *e.g.*, U.S. Patent No. 8,486,907 published on July 16, 2013 and U.S. 20140275212 published on September 18, 2014.

[000256] In SMA, there is loss of functional SMN1. Although the SMN2 gene is a paralog to SMN1, alternative splicing of the SMN2 gene predominantly leads to skipping of exon 7 and subsequent production of a truncated SMN protein that cannot compensate for SMN1 loss. In some embodiments, an oligonucleotide of the present disclosure promotes inclusion of SMN2 exon 7. In some embodiments, an oligonucleotide is an antisense oligonucleotide that targets SMN2 splice site inhibitory sequences (see, *e.g.*, US Patent Number 7,838,657, which was published on November 23, 2010).

ii. Small Molecules:

[000257] Any suitable small molecule may be used as a molecular payload, as described herein.

iii. Peptides/Proteins

[000258] Any suitable peptide or protein may be used as a molecular payload, as described herein. In some embodiments, a protein is an enzyme (e.g., an acid alpha-glucosidase, e.g., as encoded by the *GAA* gene). These 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.).

iv. Nucleic Acid Constructs

[000259] 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 protein that is deficient in a muscle disease. 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 construct encodes a gene that is deficient in a muscle disease. In some embodiments, the gene expression construct encodes a protein that comprises at least one zinc finger. In some embodiments, the gene expression construct encodes a protein that binds to a gene in Table 6. In some embodiments, the gene expression construct encodes a protein that leads to a reduction in the expression of a protein (e.g., mutant protein) encoded by a gene in Table 6. In some embodiments, the gene expression construct encodes a gene editing enzyme. Additional examples of nucleic acid constructs that may be used as molecular payloads are provided in International Patent Application Publication WO2017152149A1, published on September 19, 2017, entitled, “CLOSED-ENDED LINEAR DUPLEX DNA FOR NON-VIRAL GENE TRANSFER”; US Patent 8,853,377B2, issued on October 7, 2014, entitled, “MRNA FOR USE IN TREATMENT OF HUMAN GENETIC DISEASES”; and US Patent US8822663B2, issued on September 2, 2014, ENGINEERED

NUCLEIC ACIDS AND METHODS OF USE THEREOF,” the contents of each of which are incorporated herein by reference in their entireties.

v. Detectable labels / Diagnostic Agents

[000260] Any suitable detectable label or diagnostic agent can be used as the molecular payload of the present disclosure. A “diagnostic agent” refers to an agent that is used for diagnostic purpose, e.g., by detecting another molecule in a cell or a tissue. In some embodiments, the diagnostic agent is an agent that targets (e.g., binds) a biomarker known to be associated with a disease (e.g., a nucleic acid biomarker, protein biomarker, or a metabolite biomarker) in a subject and produces a detectable signal, which can be used to determine the presence/absence of the biomarker, thus to diagnose a disease. For example, the diagnostic agent may be, without limitation, an antibody or an antisense nucleic acid.

[000261] In some embodiments, the diagnostic agent contains a detectable label. A detectable label refers to a moiety that has at least one element, isotope, or a structural or functional group incorporated that enables detection of a molecule, e.g., a protein or polypeptide, or other entity, to which the diagnostic agent binds. In some embodiments, a detectable label falls into any one (or more) of five classes: a) an agent which contains isotopic moieties, which may be radioactive or heavy isotopes, including, but not limited to, ²H, ³H, ¹³C, ¹⁴C, ¹⁵N, ¹⁸F, ³¹P, ³²P, ³⁵S, ⁶⁷Ga, ⁷⁶Br, ^{99m}Tc (Tc-99m), ¹¹¹In, ¹²³I, ¹²⁵I, ¹³¹I, ¹⁵³Gd, ¹⁶⁹Yb, and ¹⁸⁶Re; b) an agent which contains an immune moiety, which may be an antibody or antigen, which may be bound to an enzyme (e.g., such as horseradish peroxidase); c) an agent comprising a colored, luminescent, phosphorescent, or fluorescent moiety (e.g., such as the fluorescent label fluorescein isothiocyanate (FITC); d) an agent which has one or more photo affinity moieties; and e) an agent which is a ligand for one or more known binding partners (e.g., biotin-streptavidin, His -NiTNAFK506-FKBP). In some embodiments, a detectable label comprises a radioactive isotope. In some embodiments, a detectable label comprises a fluorescent moiety. In some embodiments, the detectable label comprises a dye, e.g., a fluorescent dye, e.g., fluorescein isothiocyanate, Texas red, rhodamine, Cy3, Cy5, Cy5.5, Alexa 647 and derivatives. In some embodiments, the detectable label comprises biotin. In some embodiments, the detectable molecule is a fluorescent polypeptide (e.g., GFP or a derivative thereof such as enhanced GFP (EGFP)) or a luciferase (e.g., a firefly, Renilla, or Gaussia luciferase). In some embodiments, a detectable label may react with a suitable substrate (e.g., a luciferin) to generate a detectable signal. Non-limiting examples of fluorescent proteins include GFP and derivatives thereof, proteins comprising chromophores that emit light of different colors such as red, yellow, and cyan fluorescent proteins, etc.

Exemplary fluorescent proteins include, e.g., Sirius, Azurite, EBFP2, TagBFP, mTurquoise, ECFP, Cerulean, TagCFP, mTFP1, mUkG1, mAG1, AcGFP1, TagGFP2, EGFP, mWasabi, EmGFP, TagYPF, EYFP, Topaz, SYFP2, Venus, Citrine, mKO, mKO2, mOrange, mOrange2, TagRFP, TagRFP-T, mStrawberry, mRuby, mCherry, mRaspberry, mKate2, mPlum, mNeptune, T- Sapphire, mAmetrine, mKeima. See, e.g., Chalfie, M. and Kain, SR (eds.) *Green fluorescent protein: properties, applications, and protocols (Methods of biochemical analysis, v. 47, Wiley-Interscience, and Hoboken, N.J., 2006, and/or (e.g., and) Chudakov, DM, et al., *Physiol Rev.* 90(3):1103-63, 2010, incorporated herein by reference, for discussion of GFP and numerous other fluorescent or luminescent proteins. In some embodiments, a detectable label comprises a dark quencher, e.g., a substance that absorbs excitation energy from a fluorophore and dissipates the energy as heat.*

B. Linkers

[000262] 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.).

[000263] 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 trizole, a thioether, or a disulfide bond.

i. Cleavable Linkers

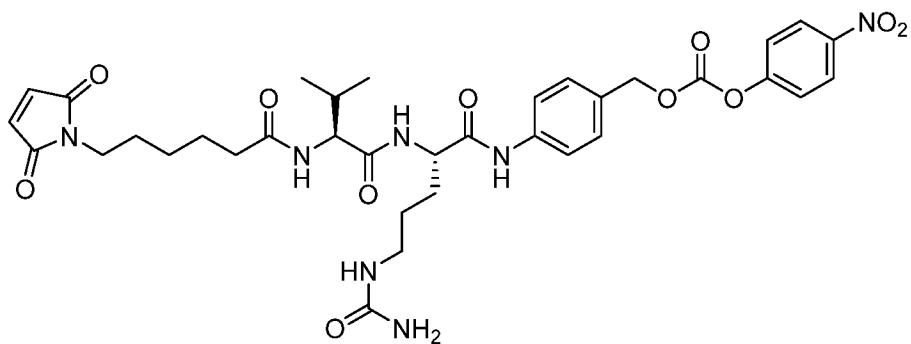
[000264] A cleavable linker may be a protease-sensitive linker, a pH-sensitive linker, or a glutathione-sensitive linker. These linkers are generally cleavable only intracellularly and are preferably stable in extracellular environments, e.g. extracellular to a muscle cell.

[000265] 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.

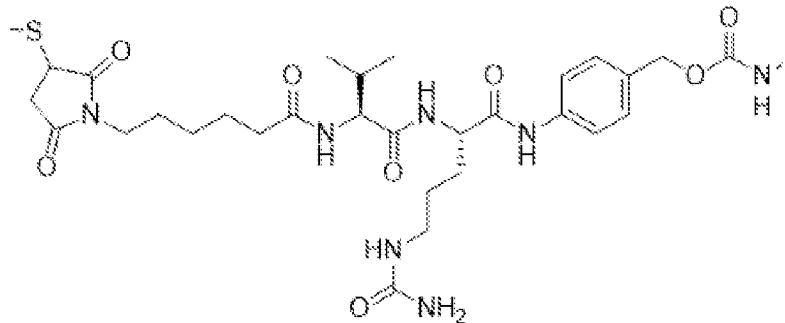
[000266] A pH-sensitive linker is a covalent linkage that readily degrades in high or low pH environments. In some embodiments, a pH-sensitive linker may be cleaved at a pH in a range of 4 to 6. In some embodiments, a pH-sensitive linker comprises a hydrazone or cyclic acetal. In some embodiments, a pH-sensitive linker is cleaved within an endosome or a lysosome.

[000267] 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.

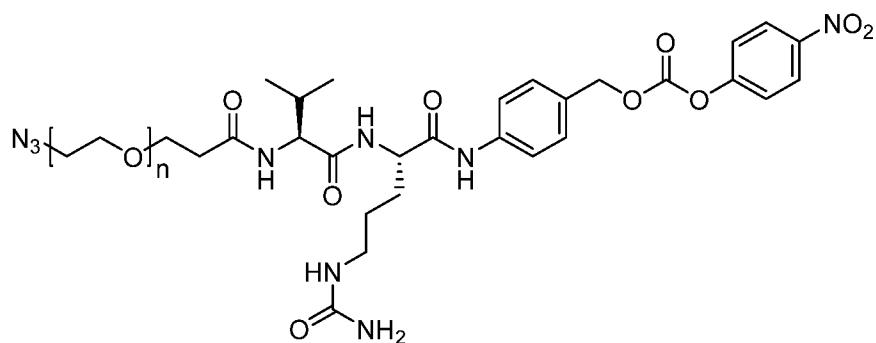
[000268] In some embodiments, the linker is a Val-cit linker (e.g., as described in US Patent 6,214,345, incorporated herein by reference). In some embodiments, before conjugation, the val-cit linker has a structure of:



[000269] In some embodiments, after conjugation, the val-cit linker has a structure of:

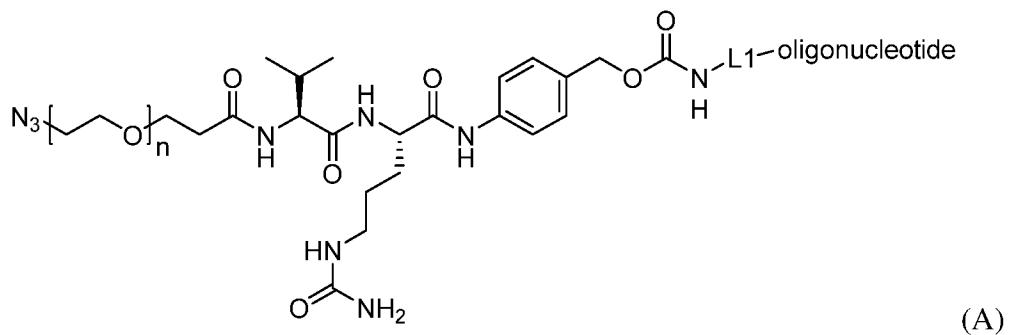


[000270] In some embodiments, the Val-cit linker is attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation). In some embodiments, before click chemistry conjugation, the val-cit linker attached to a reactive chemical moiety (e.g., SPAAC for click chemistry conjugation) has the structure of:



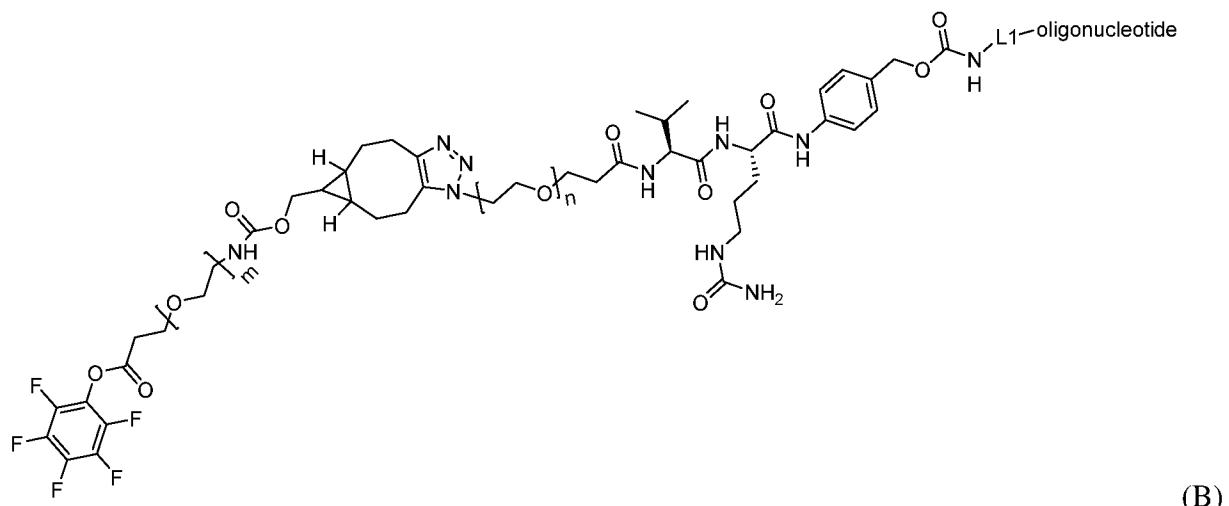
wherein n is any number from 0-10. In some embodiments, n is 3.

[000271] 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):



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

[000272] In some embodiments, after conjugation to a molecular payload (e.g., an oligonucleotide), the val-cit linker has a structure of:



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

[000273] 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.).

[000274] 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

[000275] 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.

[000276] 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*”.

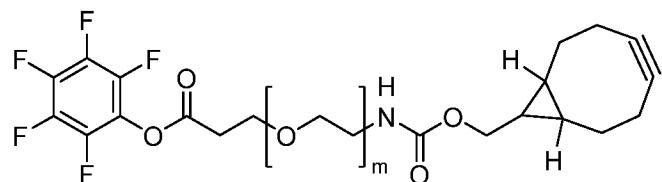
[000277] In some embodiments, a linker further comprises a spacer, e.g., a polyethylene glycol spacer or an acyl/carbomoyl sulfamide spacer, e.g., a HydraSpaceTM spacer. In some embodiments, a spacer is as described in Verkade, J.M.M. et al., “*A Polar Sulfamide Spacer Significantly Enhances the Manufacturability, Stability, and Therapeutic Index of Antibody-Drug Conjugates*”, Antibodies, 2018, 7, 12.

[000278] 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 diene/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.

[000279] In some embodiments, a linker is connected to an anti-TfR antibody and/or (e.g., and) molecular payload by a conjugate addition reaction between a nucleophile, e.g. an amine or a hydroxyl group, 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

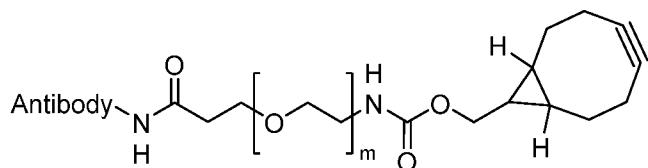
aziridine, an aryl, an activated phosphorus center, and/or (e.g., and) an activated sulfur center. In some embodiments, a nucleophile may be an optionally substituted alkene, an optionally substituted alkyne, an optionally substituted aryl, an optionally substituted heterocyclyl, a hydroxyl group, an amino group, an alkylamino group, an anilido group, or a thiol group.

[000280] 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:



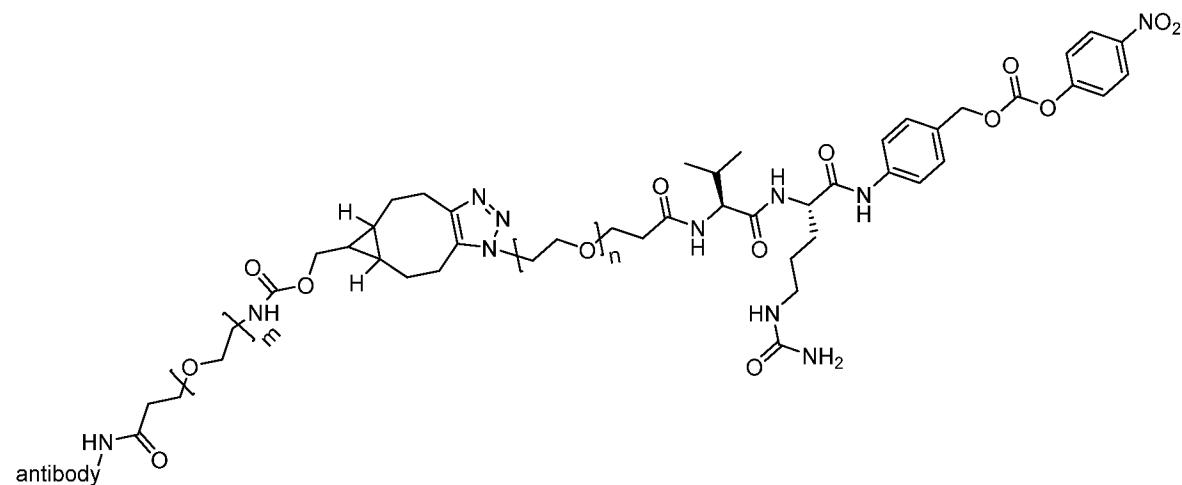
wherein m is any number from 0-10. In some embodiments, m is 4.

[000281] 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:



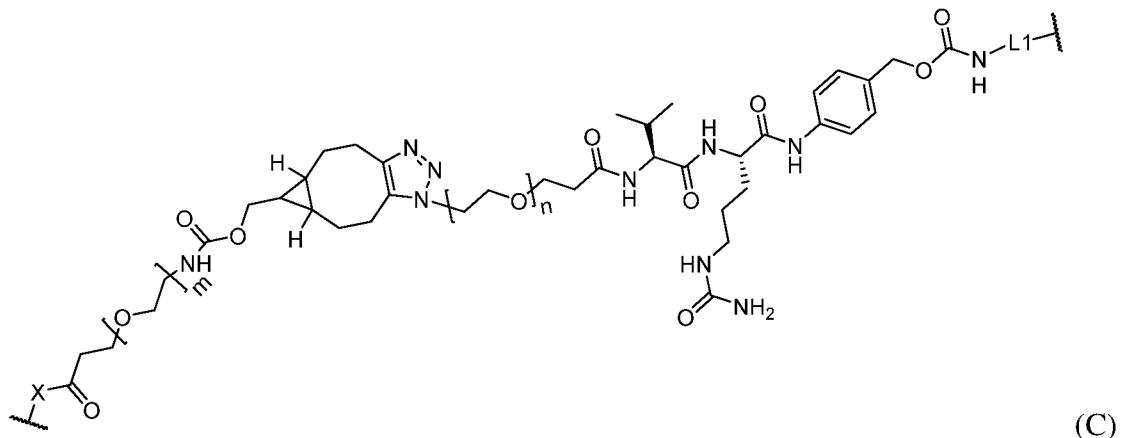
wherein m is any number from 0-10. In some embodiments, m is 4.

[000282] 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:



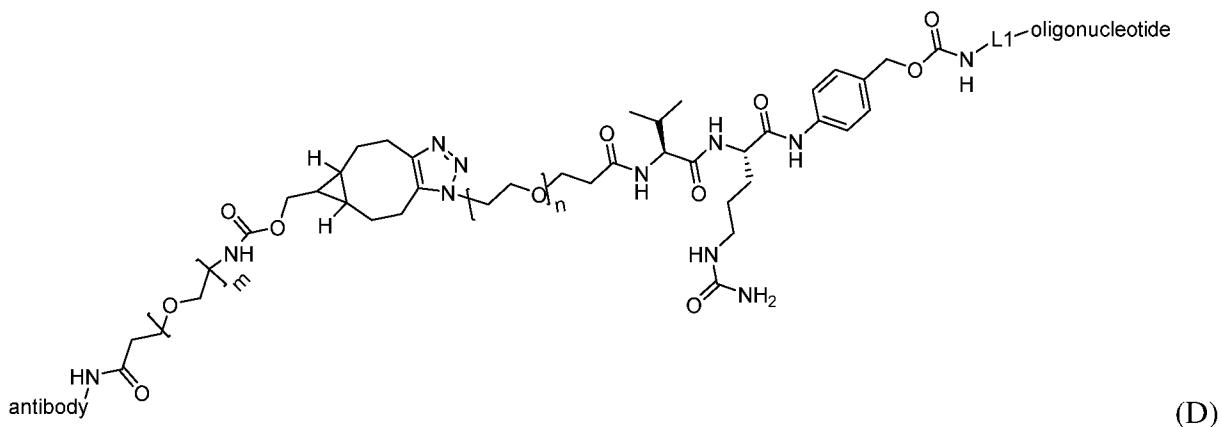
wherein n is any number from 0-10, wherein m is any number from 0-10. In some embodiments, n is 3 and/or (e.g., and) m is 4.

[000283] In some embodiments, the val-cit linker that links the antibody and the molecular payload 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. 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.

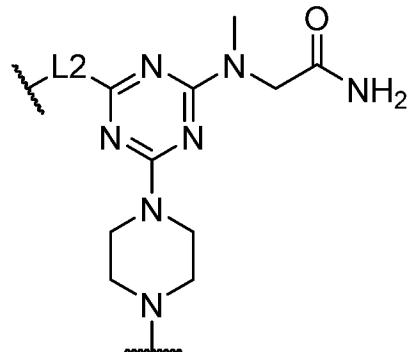
[000284] In some embodiments, the complex described herein has a structure of:



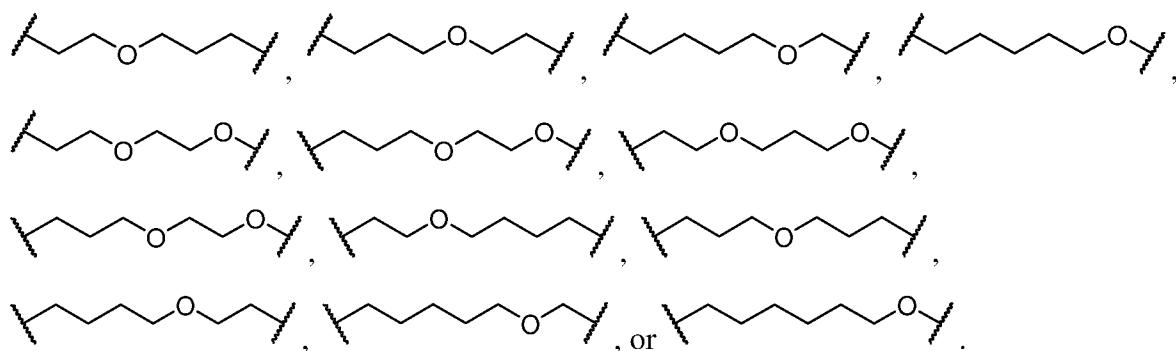
wherein n is any number from 0-10, wherein m is any number from 0-10. In some embodiments, n is 3 and/or (e.g., and) m is 4.

[000285] 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-, -

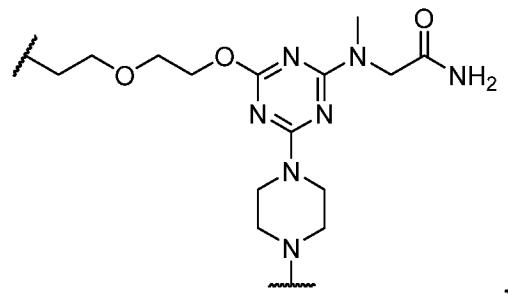
OC(=O)N(R^A)-, -S(O)₂NR^A-, -NR^AS(O)₂-, or a combination thereof. In some embodiments, L1 is



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



[000286] In some embodiments, L1 is:



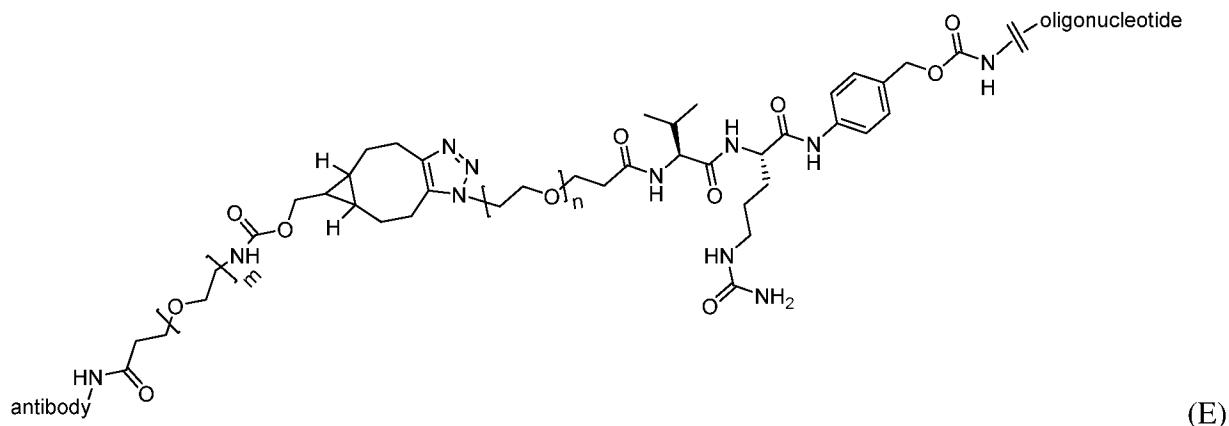
wherein the piperazine moiety links to the oligonucleotide.

[000287] In some embodiments, L1 is

[000288] In some embodiments, L1 is linked to a 5' phosphate of the oligonucleotide.

[000289] In some embodiments, L1 is optional (e.g., need not be present).

[000290] In some embodiments, any one of the complexes described herein has a structure of:

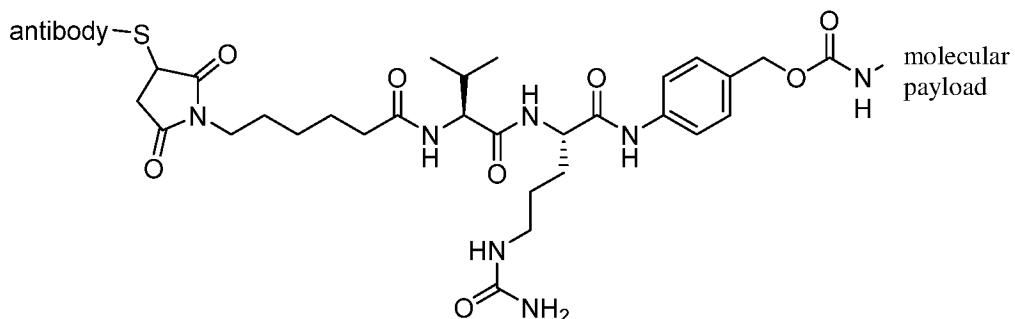


wherein n is 0-15 (e.g., 3) and m is 0-15 (e.g., 4).

C. Examples of Antibody-Molecular Payload Complexes

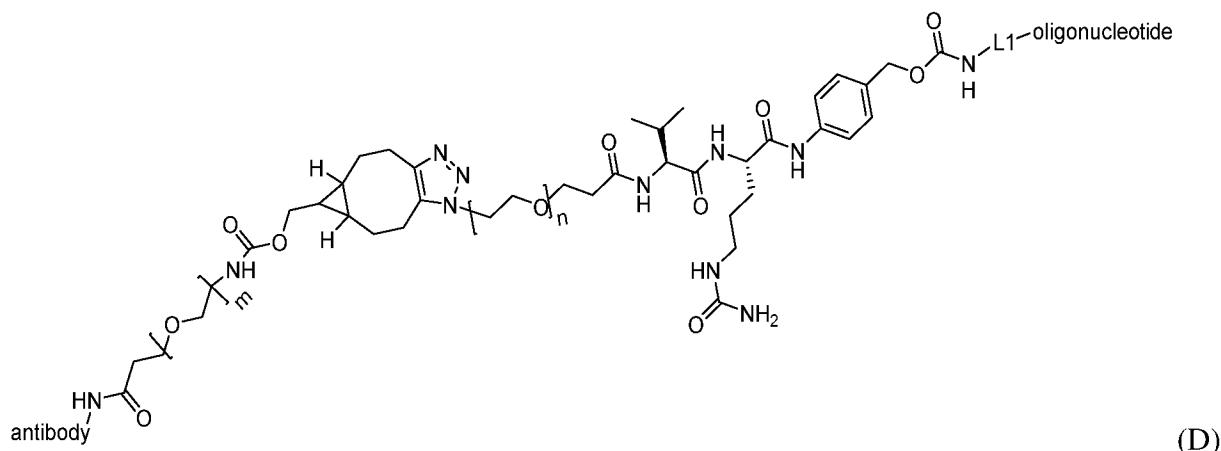
[000291] Further provided herein are non-limiting examples of complexes comprising any one the anti-TfR antibodies described herein covalently linked to any of the molecular payloads (e.g., an oligonucleotide) described herein. In some embodiments, the anti-TfR antibody (e.g., any one of the anti-TfR antibody 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).

[000292] 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).

[000293] 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:



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.

[000294] It should be appreciated that antibodies can be linked to molecular payloads with different stoichiometries, 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.

[000295] In some embodiments, the complex described herein comprises an anti-TfR antibody described herein (e.g., antibodies in Tables 2-5) covalently linked to a molecular payload. In some embodiments, the complex described herein comprises an anti-TfR antibody described herein (e.g., antibodies in Tables 2-5) 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).

[000296] In some embodiments, in any one of the examples of complexes described herein, the molecular payload is an oligonucleotide comprising a region of complementarity of at least 15 nucleotides to any one of the gene target sequences described herein.

[000297] 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.

[000298] 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.

[000299] 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.

[000300] 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.

[000301] 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.

[000302] 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.

[000303] 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.

[000304] 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.

[000305] 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.

[000306] 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.

[000307] 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.

[000308] 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.

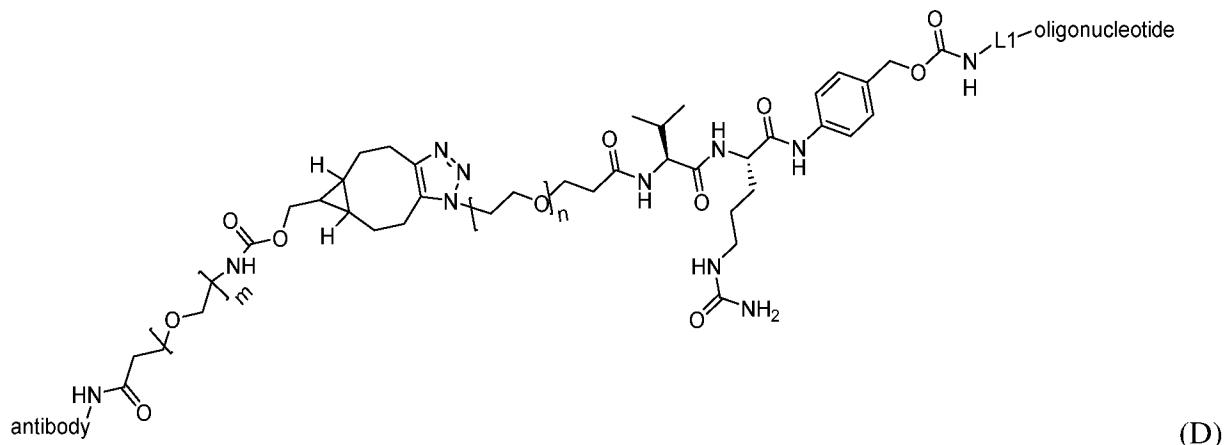
[000309] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 100 or SEQ ID NO: 101 and a light chain comprising the amino acid sequence of SEQ ID NO: 89.

[000310] 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.

[000311] 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.

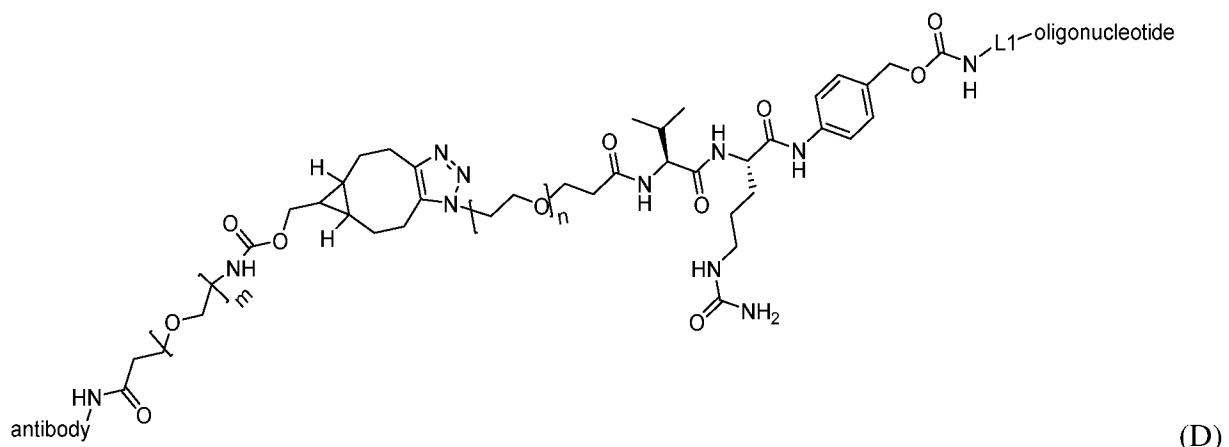
[000312] In some embodiments, the complex described herein comprises an anti-TfR antibody covalently linked to a molecular payload, wherein the anti-TfR antibody comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 or SEQ ID NO: 103 and a light chain comprising the amino acid sequence of SEQ ID NO: 95.

[000313] 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 SEQ ID NO: 85; wherein the complex has the structure of:



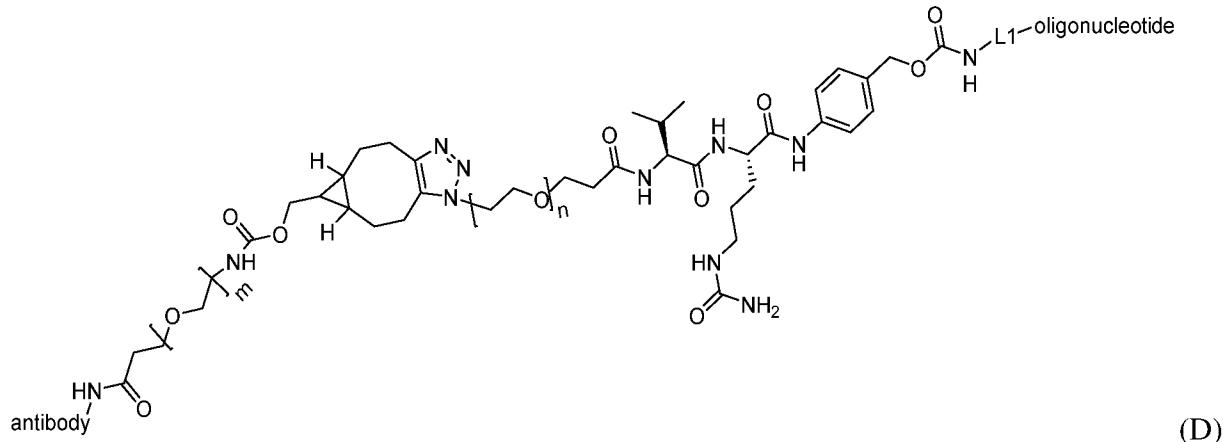
wherein n is 3 and m is 4.

[000314] 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 SEQ ID NO: 85; wherein the complex has the structure of:



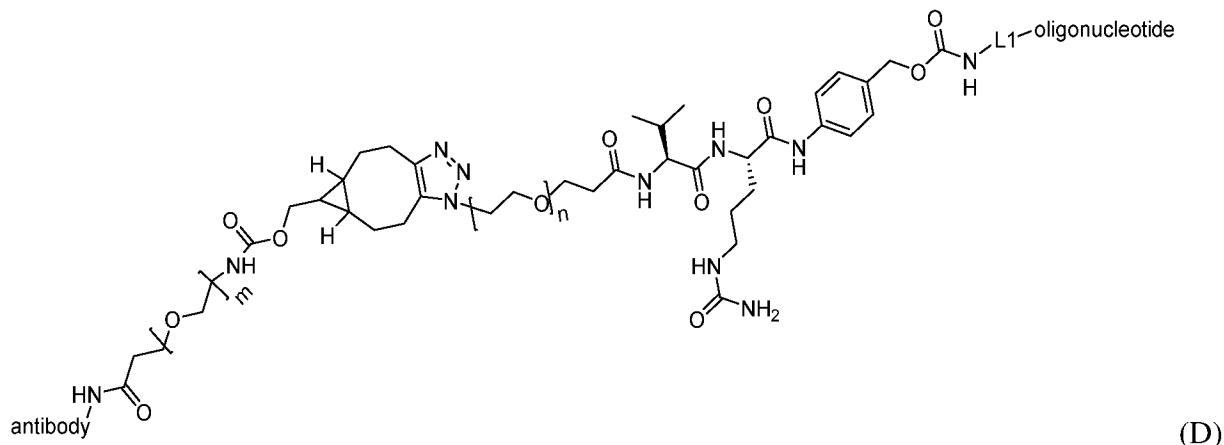
wherein n is 3 and m is 4.

[000315] 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 SEQ ID NO: 85; wherein the complex has the structure of:



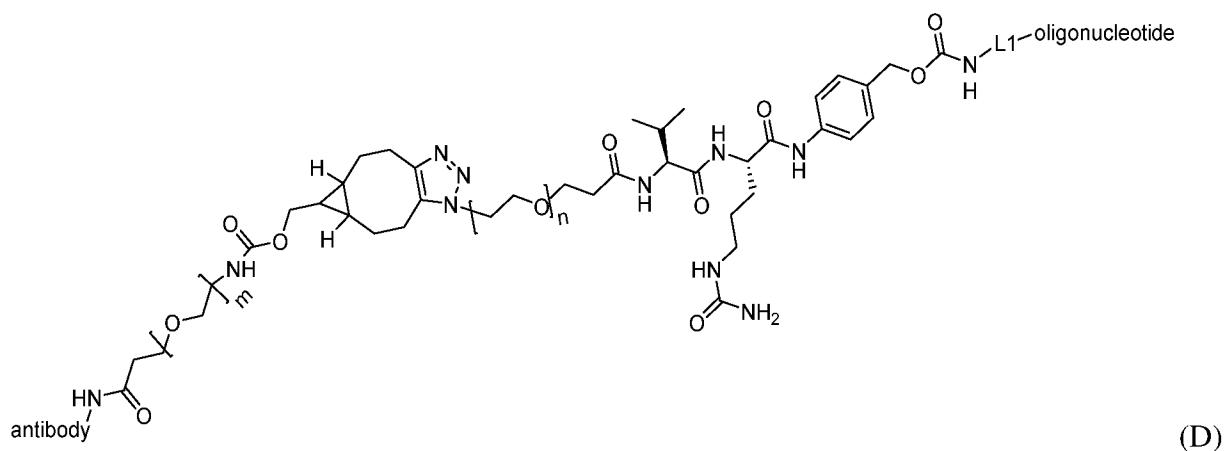
wherein n is 3 and m is 4.

[000316] 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 SEQ ID NO: 89; wherein the complex has the structure of:



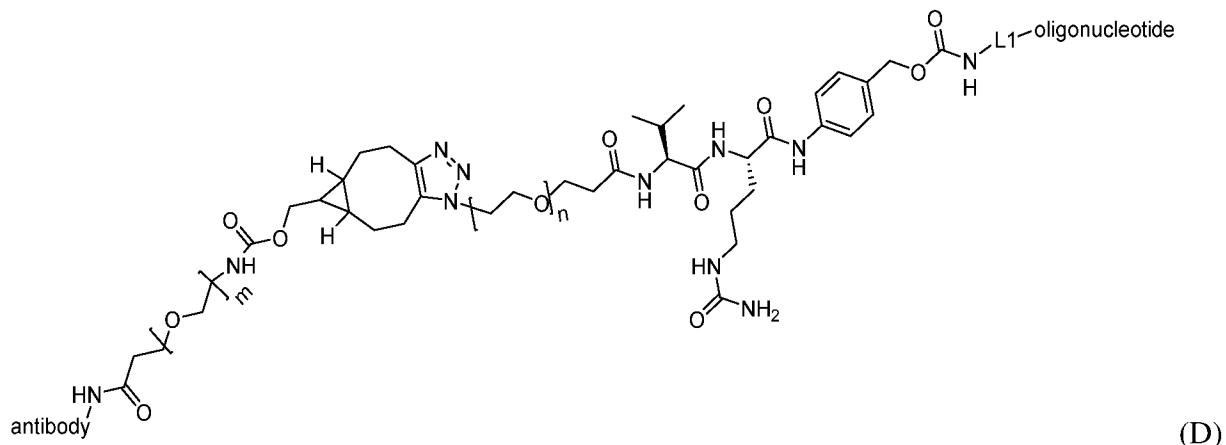
wherein n is 3 and m is 4.

[000317] 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 SEQ ID NO: 90; wherein the complex has the structure of:



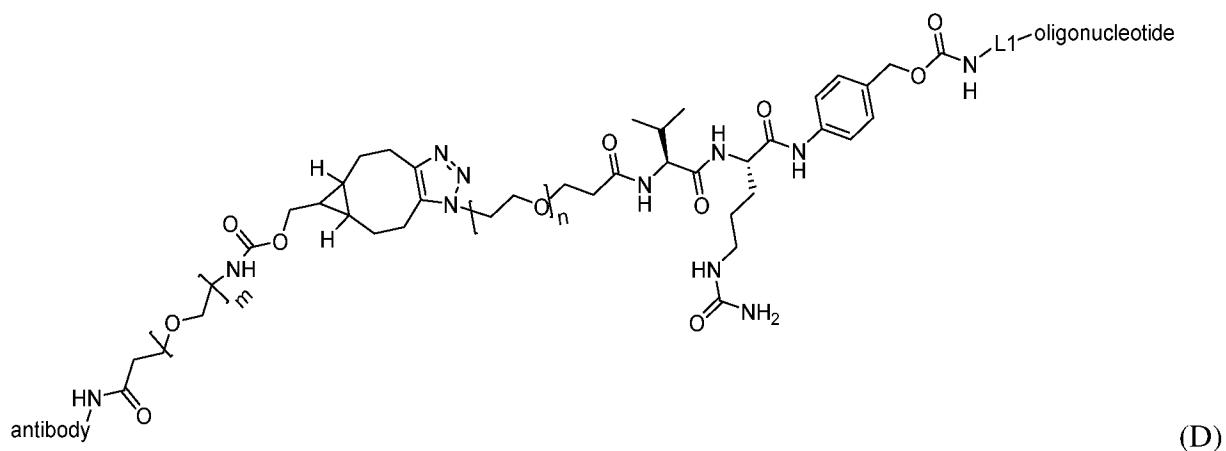
wherein n is 3 and m is 4.

[000318] 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 n SEQ ID NO: 89; wherein the complex has the structure of:



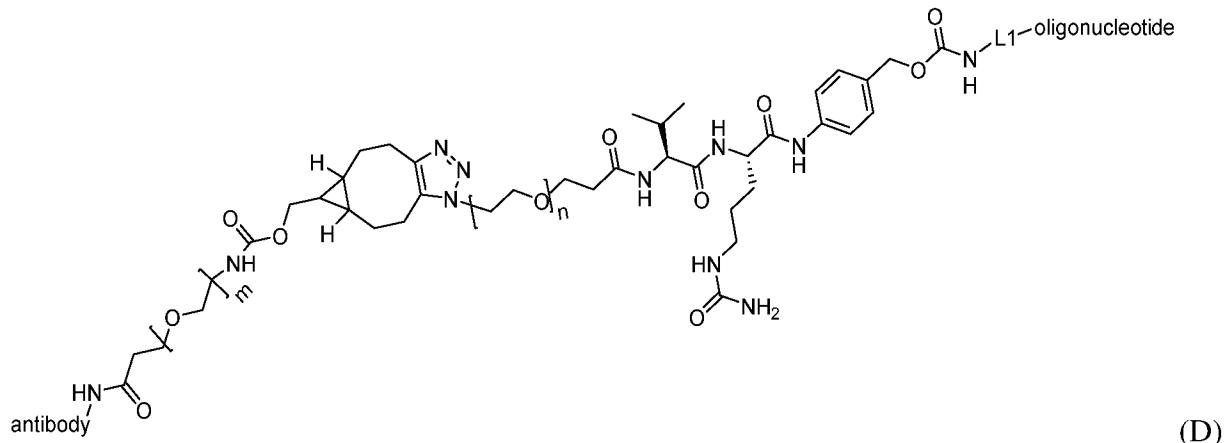
wherein n is 3 and m is 4.

[000319] 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 SEQ ID NO: 90; wherein the complex has the structure of:



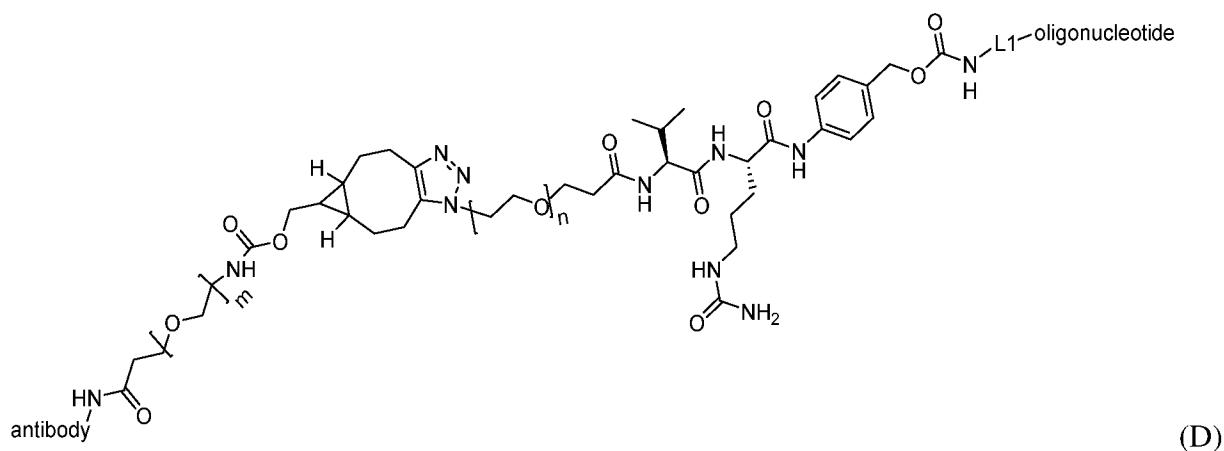
wherein n is 3 and m is 4.

[000320] 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 SEQ ID NO: 93; wherein the complex has the structure of:



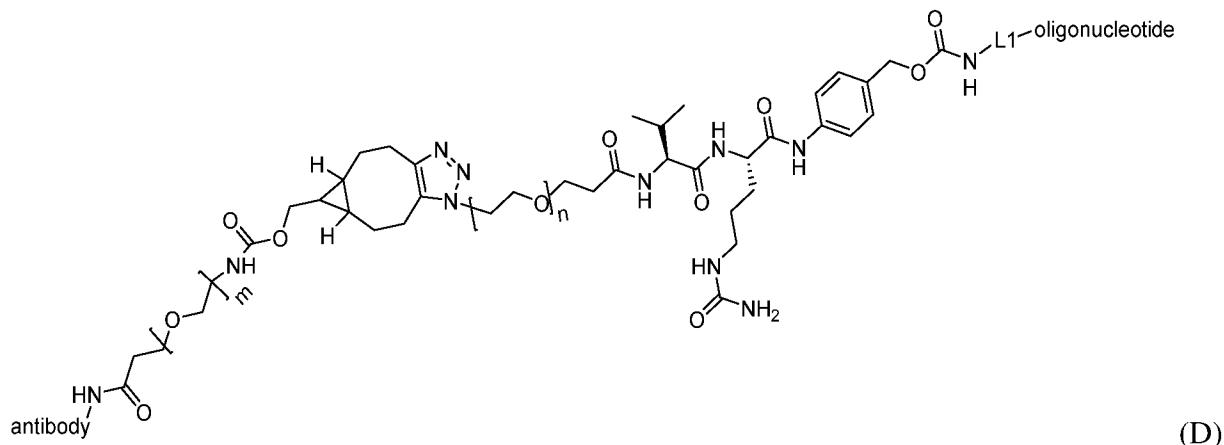
wherein n is 3 and m is 4.

[000321] 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 SEQ ID NO: 95; wherein the complex has the structure of:



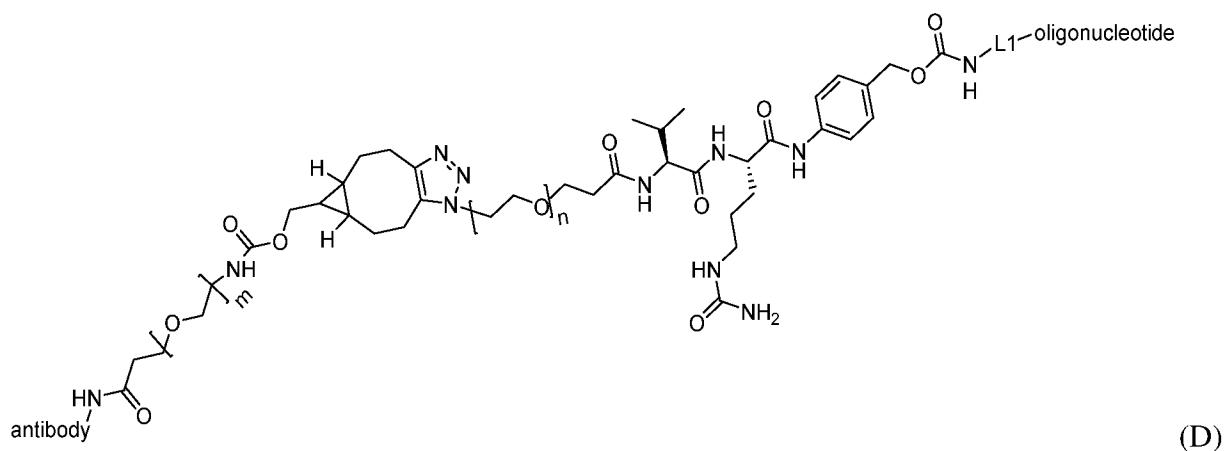
wherein n is 3 and m is 4.

[000322] 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 SEQ ID NO: 95; wherein the complex has the structure of:



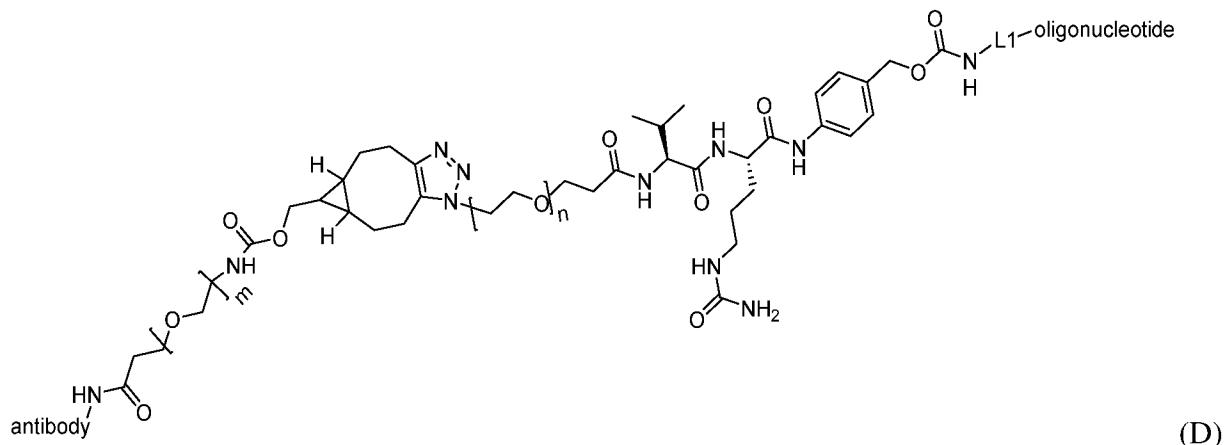
wherein n is 3 and m is 4.

[000323] 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 SEQ ID NO: 70; wherein the complex has the structure of:



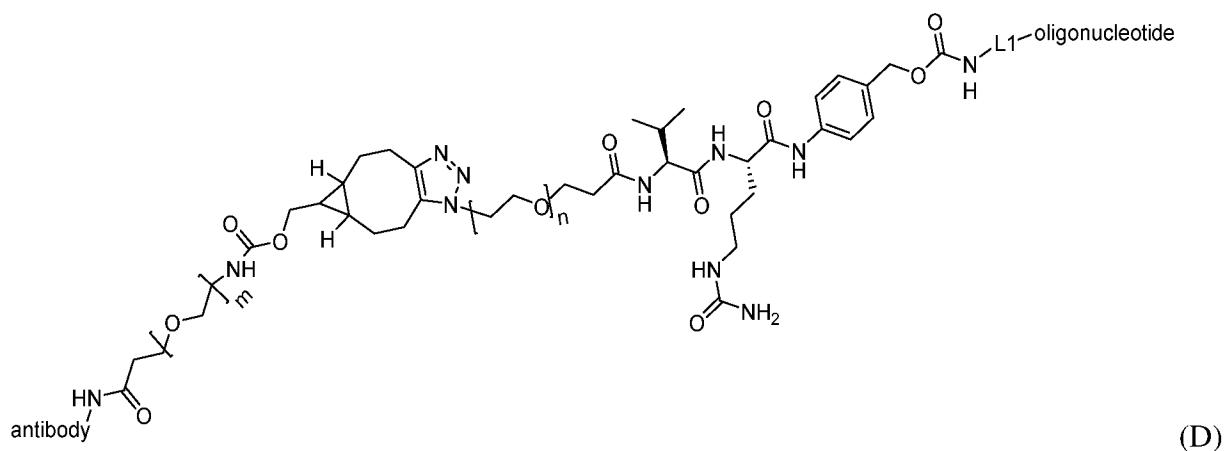
wherein n is 3 and m is 4.

[000324] 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 SEQ ID NO: 70; wherein the complex has the structure of:



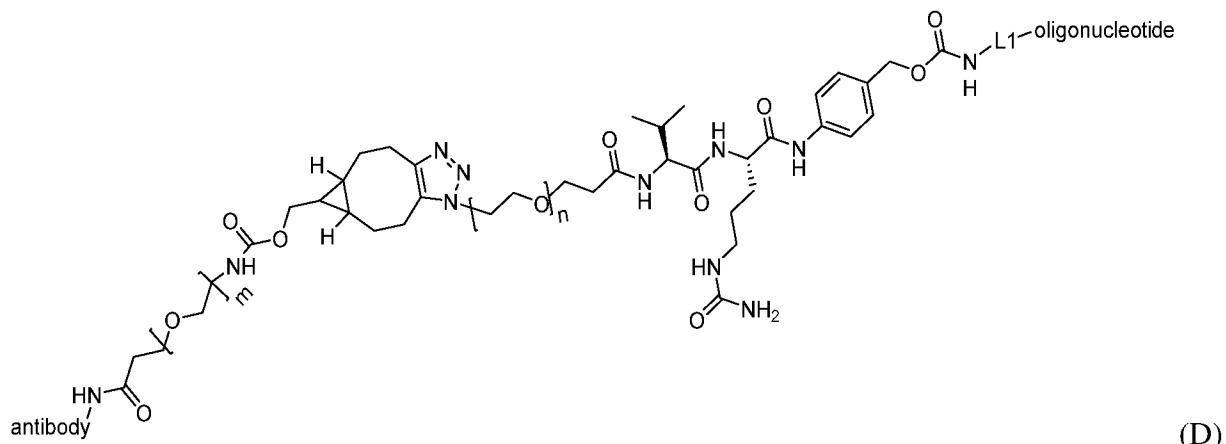
wherein n is 3 and m is 4.

[000325] 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 SEQ ID NO: 70; wherein the complex has the structure of:



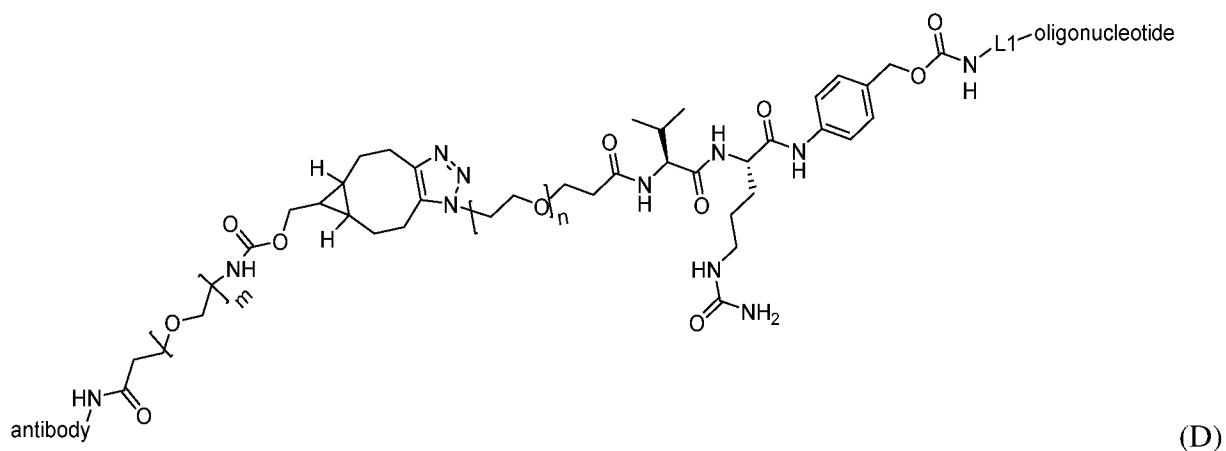
wherein n is 3 and m is 4.

[000326] 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 SEQ ID NO: 74; wherein the complex has the structure of:



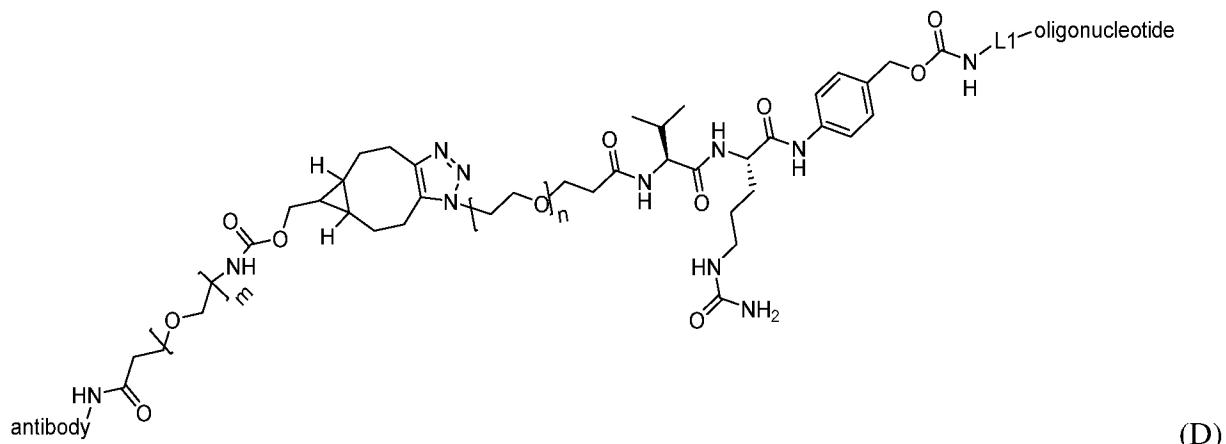
wherein n is 3 and m is 4.

[000327] 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 SEQ ID NO: 75; wherein the complex has the structure of:



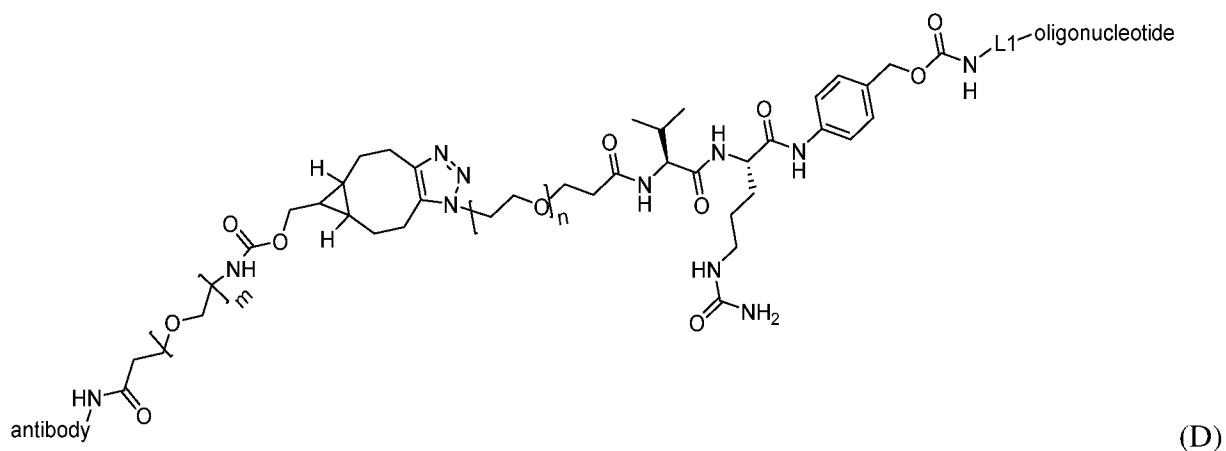
wherein n is 3 and m is 4.

[000328] 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 SEQ ID NO: 74; wherein the complex has the structure of:



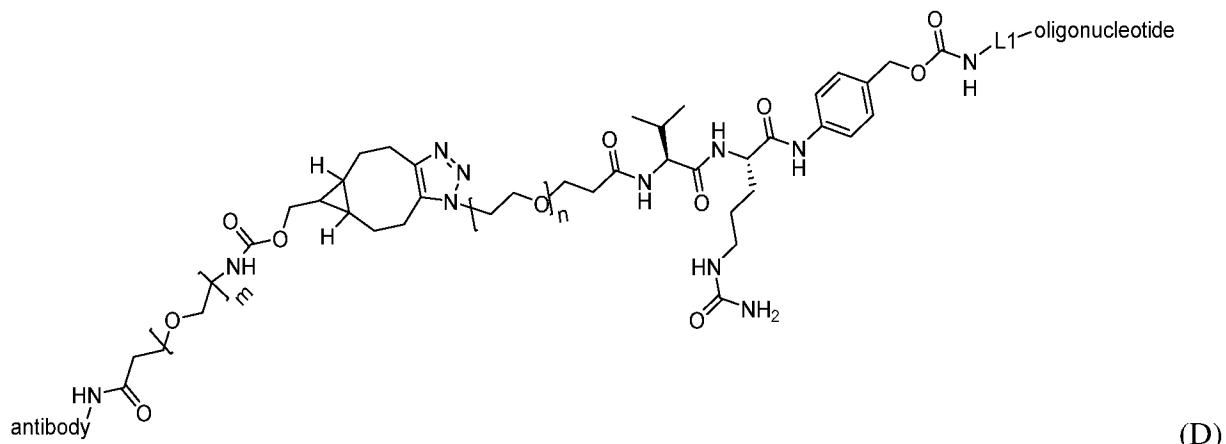
wherein n is 3 and m is 4.

[000329] 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 SEQ ID NO: 75; wherein the complex has the structure of:



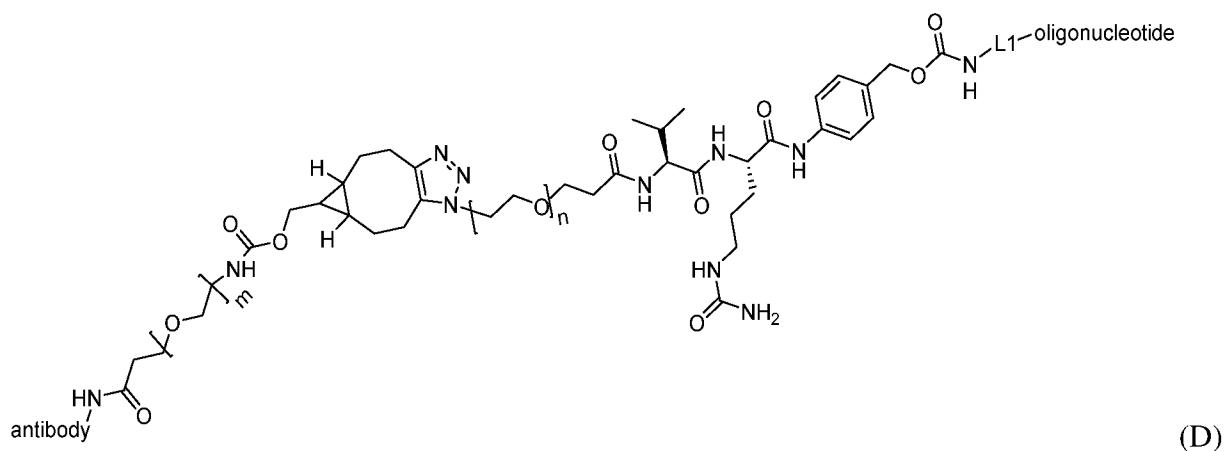
wherein n is 3 and m is 4.

[000330] 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 SEQ ID NO: 78; wherein the complex has the structure of:



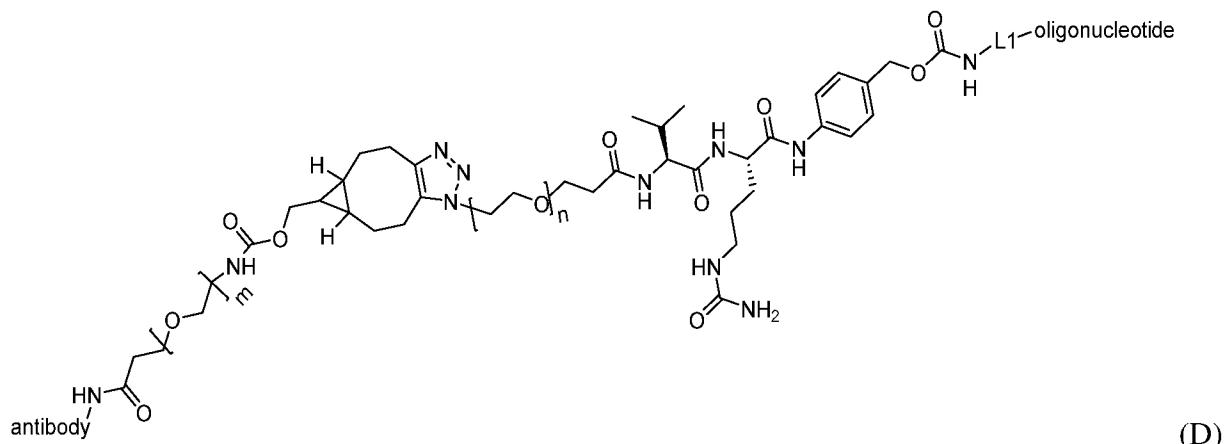
wherein n is 3 and m is 4.

[000331] 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 SEQ ID NO: 80; wherein the complex has the structure of:



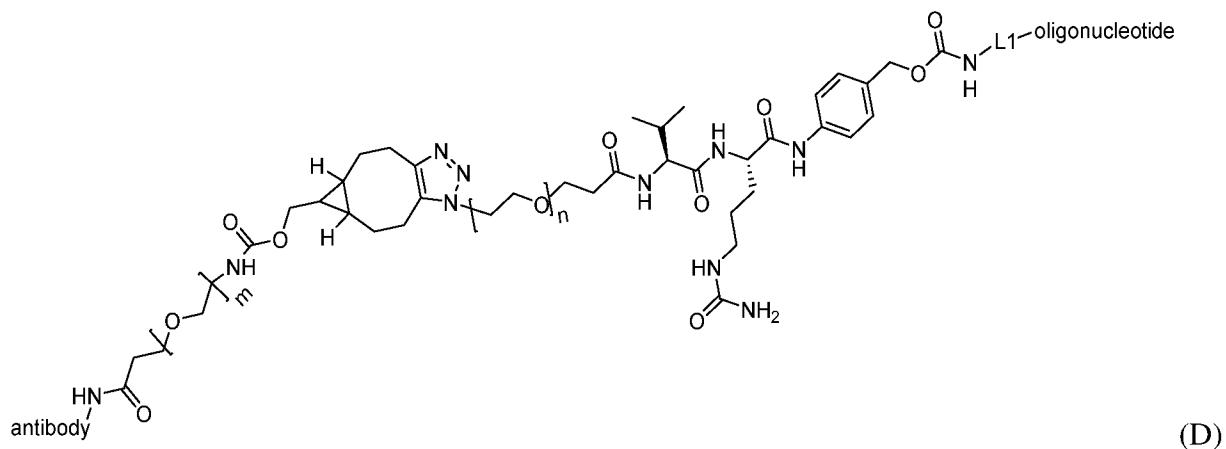
wherein n is 3 and m is 4.

[000332] 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 SEQ ID NO: 80; wherein the complex has the structure of:



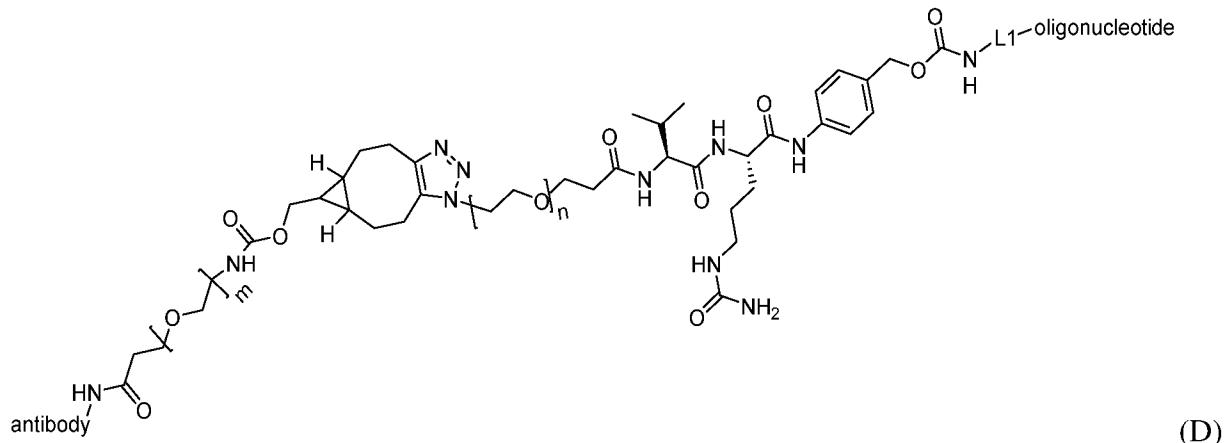
wherein n is 3 and m is 4.

[000333] 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 SEQ ID NO: 85; wherein the complex has the structure of:



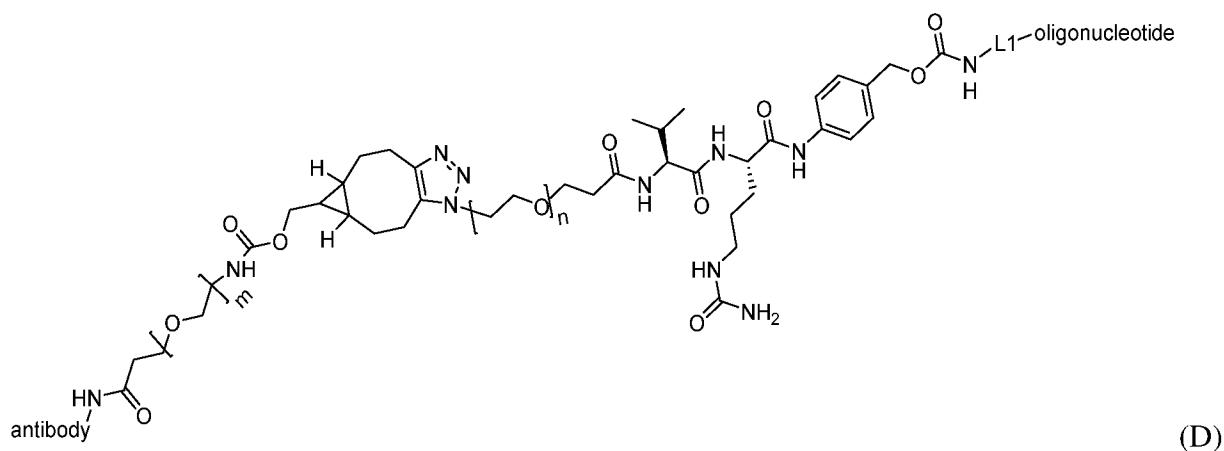
wherein n is 3 and m is 4.

[000334] 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 SEQ ID NO: 85; wherein the complex has the structure of:



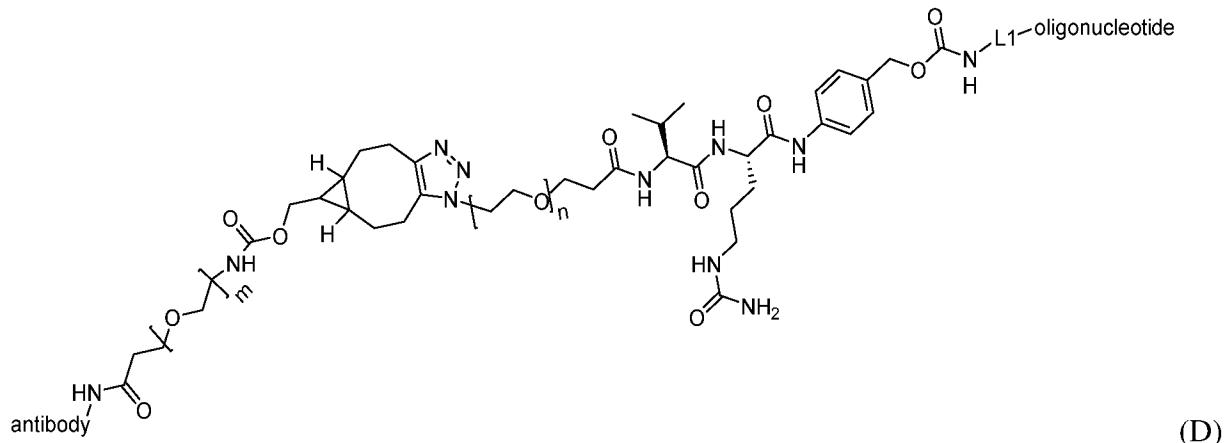
wherein n is 3 and m is 4.

[000335] 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 SEQ ID NO: 85; wherein the complex has the structure of:



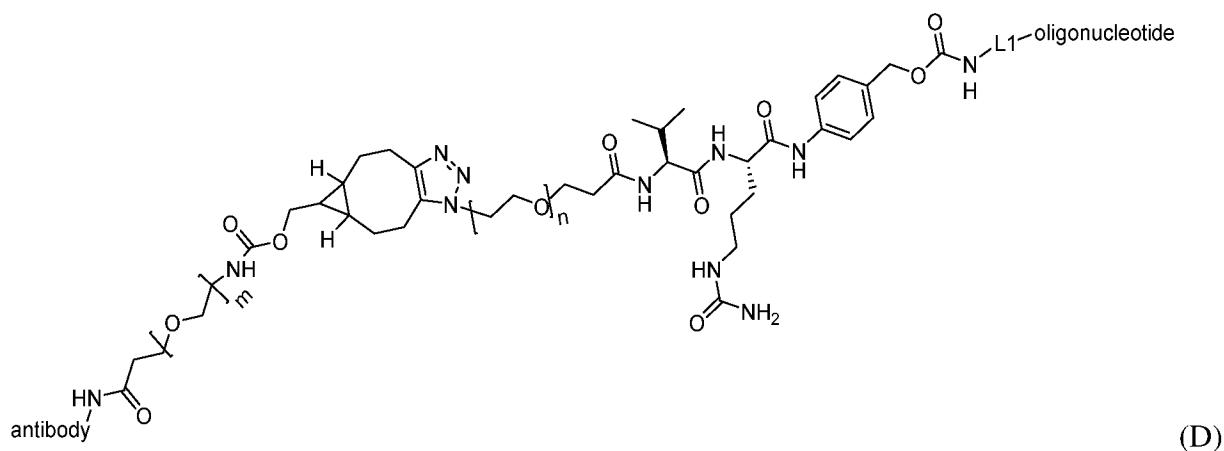
wherein n is 3 and m is 4.

[000336] 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 i SEQ ID NO: 89; wherein the complex has the structure of:



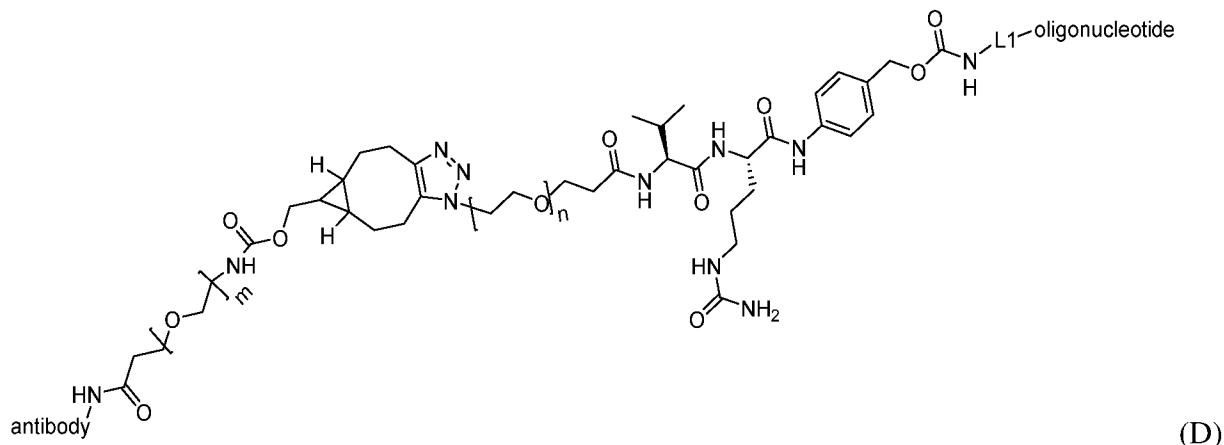
wherein n is 3 and m is 4.

[000337] 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 SEQ ID NO: 90; wherein the complex has the structure of:



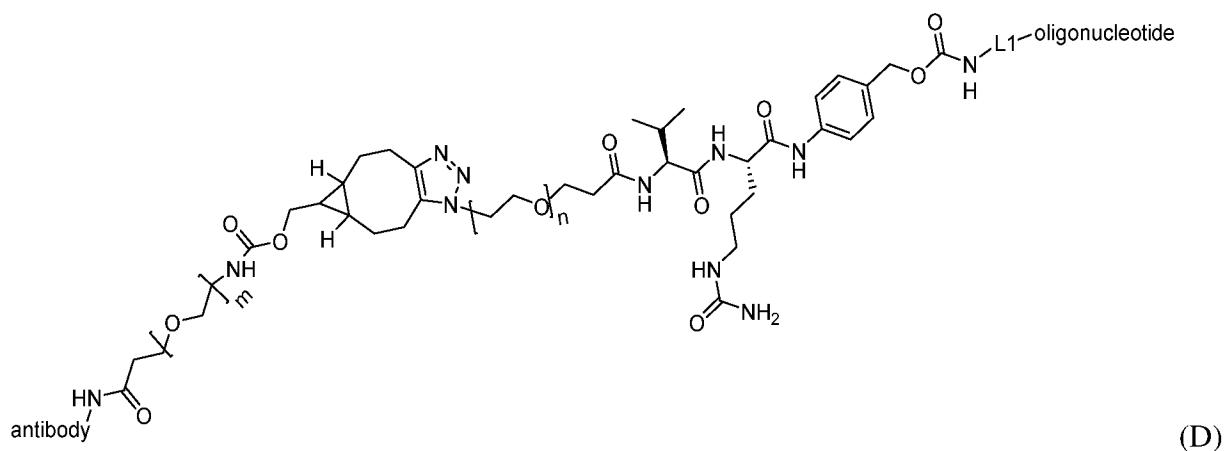
wherein n is 3 and m is 4.

[000338] 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 SEQ ID NO: 89; wherein the complex has the structure of:



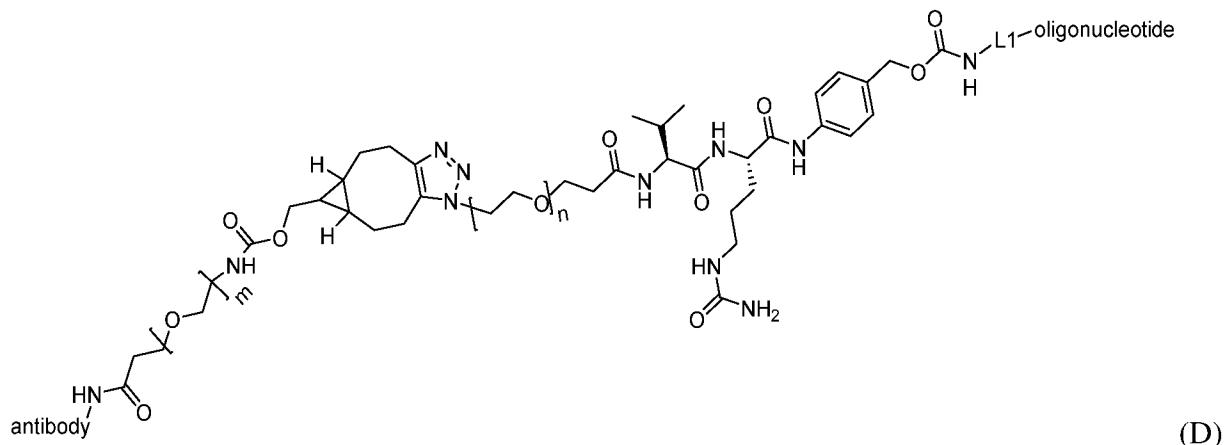
wherein n is 3 and m is 4.

[000339] 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 SEQ ID NO: 90; wherein the complex has the structure of:



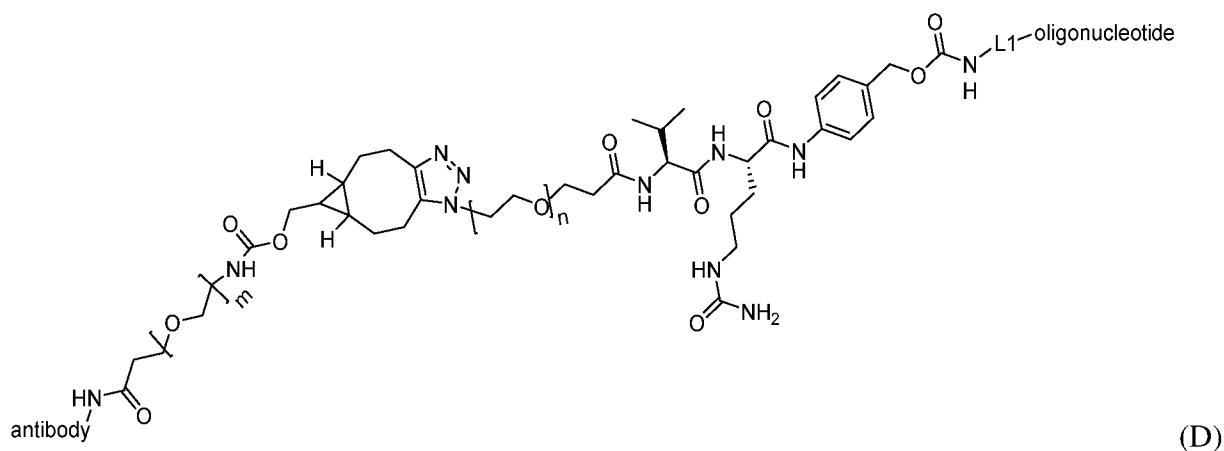
wherein n is 3 and m is 4.

[000340] 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 SEQ ID NO: 93; wherein the complex has the structure of:



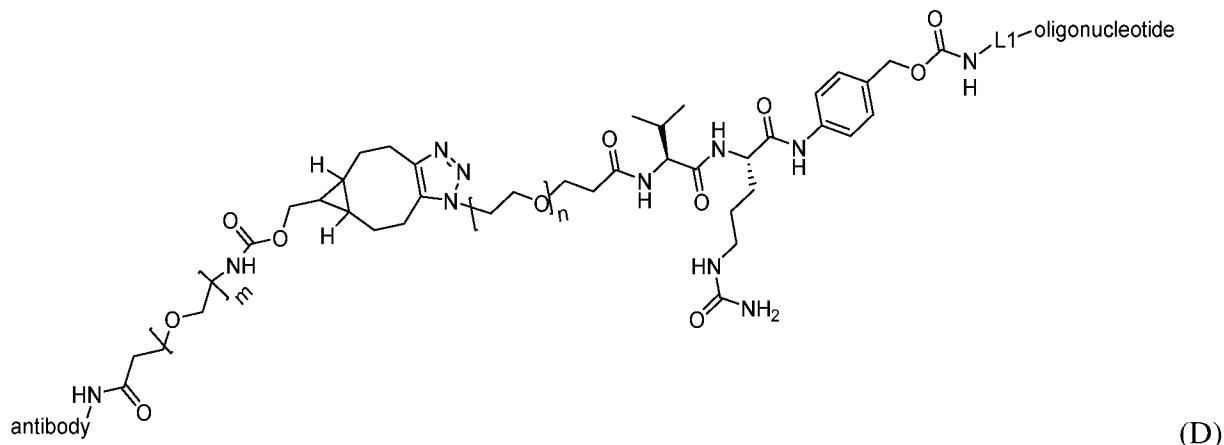
wherein n is 3 and m is 4.

[000341] 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 SEQ ID NO: 95; wherein the complex has the structure of:



wherein n is 3 and m is 4.

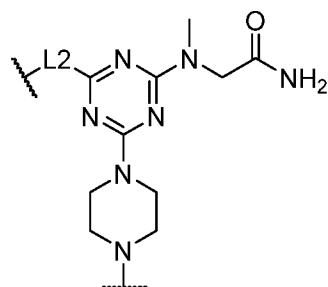
[000342] In some embodiments, the complex described herein comprises an anti-TfR Fab covalently linked via a lysine to the 5' end of an oligonucleotide, wherein the anti-TfR Fab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 102 and a light chain comprising the amino acid sequence of SEQ ID NO: 95; wherein the complex has the structure of:



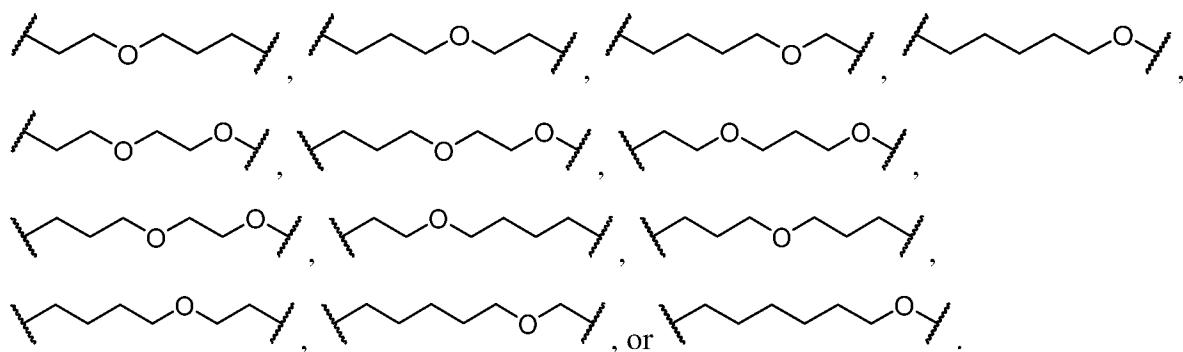
wherein n is 3 and m is 4.

[000343] In some embodiments, in any one of the examples of complexes described herein, L1 is any one of the spacers described herein.

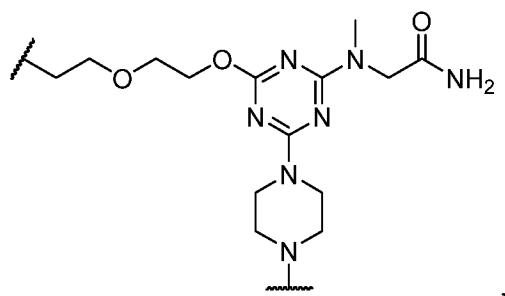
[000344] In some embodiments, L1 is:



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

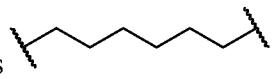


[000345] In some embodiments, L1 is:



wherein the piperazine moiety links to the oligonucleotide.

[000346] In some embodiments, L1 is



[000347] In some embodiments, L1 is linked to a 5' phosphate of the oligonucleotide.

[000348] In some embodiments, L1 is optional (e.g., need not be present).

IV. Formulations

[000349] The anti-TfR antibodies or complexes provided herein may be formulated in any suitable manner. Generally, the antibodies or complexes provided herein are formulated in a manner suitable for pharmaceutical use. For example, the antibodies or 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 the antibodies or 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, antibodies or complexes are formulated in buffer solutions such as phosphate-buffered saline solutions, liposomes, micellar structures, and capsids.

[000350] It should be appreciated that, in some embodiments, compositions may include separately one or more components of complexes provided herein (e.g., anti-TfR antibodies, linkers, molecular payloads, or precursor molecules of any one of them).

[000351] In some embodiments, antibodies or complexes are formulated in water or in an aqueous solution (e.g., water with pH adjustments). In some embodiments, antibodies or 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).

[000352] 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 pyrrolidone), or a collapse temperature modifier (e.g., dextran, ficoll, or gelatin).

[000353] 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.

[000354] 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.

[000355] In some embodiments, a composition may contain at least about 0.1% of the 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.

V. Methods of Use

[000356] Some aspects of the present disclosure provide various uses of the anti-TfR antibodies, antibody fragments or variants, nucleic acids encoding such, and complexes described herein, including in research, diagnostic methods, detection methods, and therapeutic methods. In some embodiments, the anti-TfR antibodies described herein is used for delivering a molecular payload (e.g., a diagnostic or therapeutic agent) to a target cell or tissue that expresses a transferrin receptor. In some embodiments, the target cell is a muscle cell. In some embodiments, the target tissue is muscle. In some embodiments, the target tissue is brain. For delivering the molecular payload, the anti-TfR antibody may be conjugated (e.g., covalently conjugated) to the molecular payload to form a complex.

a. Diagnostic and Detection Methods

[000357] Also provided herein are the use of any one of the above described antibodies, antigen-binding fragments, polynucleotides, vectors or cells and optionally suitable means in diagnostic and/or (e.g., and) detection methods. The antibodies or antigen-binding fragments are, for example, suited for use in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. Examples of immunoassays which can utilize the antibody or antigen-binding fragments are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the Enzyme Linked Immunoassay (ELISA), radioimmunoassay (RIA), the sandwich (immunometric assay), flow cytometry, the western blot assay, immunoprecipitation assays, immunohistochemistry, immuno-microscopy, lateral flow immuno-chromatographic assays, and proteomics arrays. The antigens and antibodies or antigen-binding fragments can be bound to many different solid supports (e.g., carriers, membrane, columns, proteomics array, etc.). Examples of well known solid support materials include glass, polystyrene, polyvinyl chloride, polyvinylidene difluoride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, such as nitrocellulose, polyacrylamides, agaroses, and magnetite. The nature of the support can be either fixed or suspended in a solution (e.g., beads).

[000358] In some embodiments, any one of the anti-TfR antibodies provided herein is useful for detecting the presence of transferrin receptor in a biological sample. The term “detecting” as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as blood, CSF, and BBB-containing tissue. The biological sample can be in vitro (e.g., cultured) or in vivo (e.g., in a subject). The present disclosure also contemplates the use of any one of the anti-TfR antibodies described herein in research use (e.g., as a reagent for immuno assays such as western blotting, immunostaining, ELISA, and/or (e.g., and) FACS).

[000359] In some embodiments, an anti-TfR antibody for use in a method of diagnosis or detection is provided. In some aspects, a method of detecting the presence of transferrin receptor in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-TfR antibody as described herein under conditions permissive for binding of the anti-TfR antibody to the transferrin receptor, and detecting whether a complex is formed between the anti-TfR antibody and the transferrin receptor. Such method may be an in vitro or in vivo method. In some embodiments, an anti-TfR antibody is used to select subjects eligible for therapy with an anti-TfR antibody, e.g. where transferrin receptor is a biomarker for selection of patients.

[000360] Exemplary disorders that may be diagnosed using an anti-TfR antibody described herein include disorders involving immature red blood cells, due to the fact that transferrin receptor is expressed in reticulocytes and is therefore detectable by any of the antibodies of the invention. Such disorders include anemia and other disorders arising from reduced levels of reticulocytes, or congenital polycythemia or neoplastic polycythemia vera, where raised red blood cell counts due to hyperproliferation of, e.g., reticulocytes, results in thickening of blood and concomitant physiological symptoms.

[000361] In some embodiments, to detect the presence/level of transferrin receptor in a biological sample, labeled anti-TfR antibodies are used. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ³²P, ¹⁴C, ¹²⁵I, ³H, and ¹³¹I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide

oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like. In some embodiments, the detectable label is an agent suitable for detecting transferrin receptor in a cell in vitro, which can be a radioactive molecule, a radiopharmaceutical, or an iron oxide particle. Radioactive molecules suitable for in vivo imaging include, but are not limited to, ¹²²I, ¹²³I, ¹²⁴I, ¹²⁵I, ¹³¹I, ¹⁸F, ⁷⁵Br, ⁷⁶Br, ⁷⁷Br, ²¹¹At, ²²⁵Ac, ¹⁷⁷Lu, ¹⁵³Sm, ¹⁸⁶Re, ¹⁸⁸Re, ⁶⁷Cu, ²¹³Bi, ²¹²Bi, ²¹²Pb, and ⁶⁷Ga. Exemplary radiopharmaceuticals suitable for in vivo imaging include ¹¹¹In Oxyquinoline, ¹³¹I Sodium iodide, ⁹⁹mTc Mebrofenin, and ⁹⁹mTc Red Blood Cells, ¹²³I Sodium iodide, ⁹⁹mTc Exametazime, ⁹⁹mTc Macroaggregate Albumin, ⁹⁹mTc Medronate, ⁹⁹mTc Mertiatide, ⁹⁹mTc Oxidronate, ⁹⁹mTc Pentetate, ⁹⁹mTc Pertechnetate, ⁹⁹mTc Sestamibi, ⁹⁹mTc Sulfur Colloid, ⁹⁹mTc Tetrofosmin, Thallium-201, or Xenon-133.

[000362] In certain embodiments, the anti-TfR antibody described herein can be used to deliver a detectable label to a target cell or tissue (e.g., muscle cell or across the blood brain barrier to the brain) for visualization of the cell or tissue (e.g., by fluorescent microscopy or by magnetic resonance imaging (MRI). Any of the detectable labels described herein can be used for this purpose.

[000363] In some embodiments, the anti-TfR antibody used in a diagnostic or detection method lacks effector function or has reduced effector function. In some embodiments, the anti-TfR antibody used in a diagnostic/detection method is engineered to have no or reduced effector function (e.g., by using a Fab, modifying the Ig backbone, introducing one or more Fc mutations reducing or eliminating effector function, and/or (e.g., and) modifying the glycosylation state of the antibody).

[000364] Various techniques are available for determining binding of the antibody to the transferrin receptor. One such assay is an enzyme linked immunosorbent assay (ELISA) for confirming an ability to bind to human transferrin receptor (and brain antigen). According to this assay, plates coated with antigen (e.g. recombinant transferrin receptor) are incubated with a sample comprising the anti-TfR antibody and binding of the antibody to the antigen of interest is determined.

[000365] To perform a diagnostic assay in vivo, a suitable amount of anti-TfR antibodies, conjugated with a label (e.g., an imaging agent or a contrast agent), can be administered to a subject in need of the examination. Presence of the labeled antibody can be detected based on the signal released from the label by routine methods. Assays for evaluating uptake of

systemically administered antibody and other biological activity of the antibody are known to those skilled in the art.

[000366] To perform scientific research assays, an anti-TfR antibody can be used to study bioactivity of transferrin receptor and/or (e.g., and) detect the presence of transferrin receptor intracellularly. For example, a suitable amount of anti-TfR antibody can be brought in contact with a sample (e.g. a new cell type that is not previously identified as transferrin receptor producing cells) suspected of producing transferrin receptor. The antibody and the sample may be incubated under suitable conditions for a suitable period to allow for binding of the antibody to the transferrin receptor antigen. Such an interaction can then be detected via routine methods, e.g., ELISA, histological staining or FACS.

b. Treatment Methods

[000367] The anti-TfR antibodies described herein can be used for delivering molecular payloads that are therapeutic agents (e.g., oligonucleotides, peptides/proteins, nucleic acid constructs, etc.). In some aspects, the present disclosure also provides complexes comprising the anti-TfR antibodies covalently linked to a molecular payload for use in treating diseases.

[000368] In some aspects, complexes comprising an anti-TfR antibody covalently linked to a molecular payload as described herein are effective in treating a muscle disease (e.g., a rare muscle disease or muscle atrophy). In some embodiments, complexes are effective in treating a rare muscle disease provided in Table 6. In some embodiments, a muscle disease is associated with a disease allele, for example, a disease allele for a particular muscle disease may comprise a genetic alteration in a corresponding gene listed in Table 6.

[000369] In some embodiments, complexes are effective in treating muscle atrophy associated with the activity of one or more genes listed in Table 6 under the “Muscle Atrophy Gene Targets” section. In some embodiments, muscle atrophy is due to a chronic illness, including AIDS, congestive heart failure, cancer, chronic obstructive pulmonary disease, and renal failure, or muscle disuse.

[000370] In other aspects, complexes comprising an anti-TfR antibody covalently linked to a molecular payload as described herein are effective in treating a neurological disease. In some embodiments, neurological diseases include, but are not limited to, neuropathy, amyloidosis, cancer, an ocular disease or disorder, viral or microbial infection, inflammation, ischemia, neurodegenerative disease, seizure, behavioral disorders, and a lysosomal storage disease. For the purposes of this application, the CNS will be understood to include the eye, which is normally sequestered from the rest of the body by the blood-retina barrier. Specific

examples of neurological disorders include, but are not limited to, neurodegenerative diseases (including, but not limited to, Lewy body disease, postpoliomyelitis syndrome, Shy-Draeger syndrome, olivopontocerebellar atrophy, Parkinson's disease, multiple system atrophy, striatonigral degeneration, tauopathies (including, but not limited to, Alzheimer disease and supranuclear palsy), prion diseases (including, but not limited to, bovine spongiform encephalopathy, scrapie, Creutzfeldt-Jakob syndrome, kuru, Gerstmann-Straussler-Scheinker disease, chronic wasting disease, and fatal familial insomnia), bulbar palsy, motor neuron disease, and nervous system heterodegenerative disorders (including, but not limited to, Canavan disease, Huntington's disease, neuronal ceroid-lipofuscinosis, Alexander's disease, Tourette's syndrome, Menkes kinky hair syndrome, Cockayne syndrome, Halervorden-Spatz syndrome, lafora disease, Rett syndrome, hepatolenticular degeneration, Lesch-Nyhan syndrome, and Unverricht-Lundborg syndrome), dementia (including, but not limited to, Pick's disease, and spinocerebellar ataxia), cancer (e.g. of the CNS, including brain metastases resulting from cancer elsewhere in the body). In some embodiments, for treating a neurological disease, the complex comprises an anti-TfR antibody described herein conjugated to a drug for treating a neurological disease (e.g., the drugs listed in Table 7).

[000371] 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 a muscle disease provided in Table 6. In some embodiments, a subject may have muscle atrophy, or be at risk of developing muscle atrophy.

[000372] An aspect of the disclosure includes a method 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 an anti-TfR antibody 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.

[000373] Compositions for intravenous administration may contain various carriers such as vegetable oils, dimethylactamide, dimethylformamide, 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.

[000374] In some embodiments, a pharmaceutical composition that comprises a complex comprising an anti-TfR antibody 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.

[000375] In some embodiments, a pharmaceutical composition that comprises a complex comprising an anti-TfR antibody 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.

[000376] 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.

[000377] 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

[000378] 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 muscle disease and/or (e.g., and) muscle atrophy.

[000379] In some embodiments, a pharmaceutical composition that comprises a complex comprising an anti-TfR antibody covalently linked to a molecular payload described herein is administered to a subject at an effective concentration sufficient to inhibit 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.

[000380] In some embodiments, a single dose or administration of a pharmaceutical composition that comprises a complex comprising an anti-TfR antibody 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 an anti-TfR antibody 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 an anti-TfR antibody 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.

[000381] In some embodiments, a pharmaceutical composition may comprise more than one complex comprising an anti-TfR antibody 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 muscle disease (e.g., a muscle disease provided in Table 6). 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.

c. Kits for Therapeutic and Diagnostic Applications

[000382] The present disclosure also provides kits for the therapeutic or diagnostic

applications as disclosed herein. Such kits can include one or more containers comprising an anti-TfR antibody, e.g., any of those described herein.

[000383] In some embodiments, the kit can comprise instructions for use in accordance with any of the methods described herein. The included instructions can comprise a description of administration of the anti-TfR antibody to treat, delay the onset, or alleviate a target disease as those described herein. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the target disease. In still other embodiments, the instructions comprise a description of administering an antibody to an individual at risk of the target disease.

[000384] The instructions relating to the use of an anti-TfR antibody generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

[000385] The label or package insert indicates that the composition is used for treating, delaying the onset and/or (e.g., and) alleviating a disease or disorder treatable by modulating immune responses, such as autoimmune diseases. Instructions may be provided for practicing any of the methods described herein.

[000386] The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like.

[000387] Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-TfR antibody as those described herein.

[000388] Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container. In some embodiments, the invention provides

articles of manufacture comprising contents of the kits described above.

[000389] Also provided herein are kits for use in detecting transferrin receptor in a sample. Such a kit may comprise any of the anti-TfR antibodies described herein. In some instances, the anti-TfR antibody can be conjugated with a detectable label as those described herein. As used herein, “conjugated” or “attached” means two entities are associated, preferably with sufficient affinity that the therapeutic/diagnostic benefit of the association between the two entities is realized. The association between the two entities can be either direct or via a linker, such as a polymer linker. Conjugated or attached can include covalent or noncovalent bonding as well as other forms of association, such as entrapment, e.g., of one entity on or within the other, or of either or both entities on or within a third entity, such as a micelle.

[000390] Alternatively or in addition (e.g., in addition), the kit may comprise a secondary antibody capable of binding to anti-TfR antibody. The kit may further comprise instructions for using the anti-TfR antibody for detecting transferrin receptor.

EXAMPLES

Example 1: Humanized anti-TfR1 antibodies

[000391] 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 Biacore 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).

[000392] 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 8** and **Table 9**. The selected Fabs were analyzed for their properties binding to TfR1 by kinetic analysis. The results of these analyses are shown in **Table 10**. For conjugates shown in Table 8 and Table 9, 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 10).

Table 8. 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 9. 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 10. 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}	Chi² (RU²)
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)

[000393] 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 μ L/well of recombinant huTfR1 at 1 μ g/mL in PBS and incubated at 4°C overnight. Wells were emptied and residual liquid was removed. Blocking was conducted by adding 200 μ L 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 μ L of TBST. Anti-TfR1 antibodies were then added in 0.5% BSA/TBST in triplicate in an 8 point serial dilution (dilution range 5 μ g/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 μ L of TBST. Anti-(H+L)IgG-A488 (1:500) (Invitrogen

#A11013) was diluted in 0.5% BSA in TBST, and 100 μ L 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 μ L 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 EC₅₀. The data for binding to human TfR1 (hTfR1) for the humanized 3M12, 3A4 and 5H12 Fabs are shown in **FIG. 1A**, **1C**, and **1E**, 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. 1B**, **1D**, and **1F**.

[000394] 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.

[000395] 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.

[000396] 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. 2, 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.

[000397] Conjugates of six humanized anti-TfR Fabs 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. 3A** and **3B**; 5H12, **FIG. 3E** and **3F**). Interestingly, 3A4 clones show improved binding to cTfR1 after conjugation relative to their unconjugated forms (**FIG. 3C** and **3D**).

[000398] As used in this Example, the term ‘unconjugated’ indicates that the antibody was not conjugated to an oligonucleotide.

Example 2. Knockdown of DMPK mRNA level facilitated by antibody-oligonucleotide conjugates *in vitro*

[000399] Conjugates containing humanized anti-TfR Fabs 3M12(VH3/Vk2), 3M-12 (VH4/Vk3), and 3A4(VH3-N54S/Vk4) were conjugated to a DMPK-targeting antisense 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).

[000400] 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.

[000401] Results in **FIG. 4** 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.

Example 3. Serum stability of the linker linking the anti-TfR antibody and the molecular payload

[000402] 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.

[000403] FIG. 5 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 4. Exon-skipping activity of anti-TfR conjugates in DMD patient myotubes

[000404] In this study, the exon-skipping activities of anti-TfR conjugates containing an anti-TfR Fab (3M12 VH3/VK2, 3M12 VH4/VK3, and 3A4 VH3 N54S/VK4) conjugated to a DMD exon51-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 confluence. 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 μ M. 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:

$$\% \text{Exon Skipping} = \frac{\text{Skipped Amplicon}}{(\text{Skipped Amplicon} + \text{Unskipped Amplicon})} * 100$$

[000405] The data demonstrates that the conjugates with either 3M12 VH3/V κ 2 or 3M12 VH4/V κ 3 Fab conjugated to the DMD exon 51-skipping oligonucleotide resulted in enhanced

exon skipping compared to the unconjugated DMD exon skipping oligonucleotide in patient myotubes (FIG. 6).

[000406] As used in this Example, the term ‘unconjugated’ indicates that the oligonucleotide was not conjugated to an antibody.

Example 5. *In vivo* activity of anti-TfR conjugates in hTfR1 mice

[000407] In DM1, the higher than normal number of CUG repeats form large hairpin loops that remain trapped in the nucleus, forming nuclear foci that bind splicing proteins and inhibit the ability of splicing proteins to perform their normal function. When toxic nuclear DMPK levels are reduced, the nuclear foci are diminished, releasing splicing proteins, allowing restoration of normal mRNA processing, and potentially stopping or reversing disease progression.

[000408] The *in vivo* activity of conjugates containing an anti-TfR Fab (control, 3M12 VH3/VK2, 3M12 VH4/VK3, 3A4 VH3 N54S/VK4) conjugated to the DMPK-targeting oligonucleotide ASO300 in reducing DMPK mRNA level in multiple muscle tissues following systemic intravenous administration in mice was evaluated.

[000409] Male and female C57BL/6 mice where one TfR1 allele was replaced with a human TFR1 allele were administered between the ages of 5 and 15 weeks according to the dosing schedule outlined in Table 11 and in FIG. 7A. Mice were sacrificed 14 days after the first injection and selected muscles collected as indicated in Table 12.

Table 11

Group	Animal No.	Treatment Antibody	Treatment Oligo	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dosing Regimen	Terminal Time Point
1	4	Vehicle	NA	0 5.0 10.2 11.5 10.1 10.7	10	Day 0 and Day 7 by IV	Day 14
2	4	NA	ASO300		5.0		
3	4	control anti-TfR Fab	ASO300		10.2		
4	4	3M12 VH3/VK2	ASO300		11.5		
5	4	3M12 VH4/VK3	ASO300		10.1		
6	4	3A4 VH3 N54S/VK4	ASO300		10.7		

Table 12

Tissue	Storage
Gastrocnemius	Right leg of each animal stored in RNAlater at -80 °C
Tibialis Anterior	One leg (R) of each animal stored in RNAlater at -80 °C
Heart	Dissect transversally and store the apex in RNAlater at -80 °C
Diaphragm	Split in half and collect one half in RNAlater at -80 °C

[000410] Total RNA was extracted on a Maxwell Rapid Sample Concentrator (RSC) Instrument using kits provided by the manufacturer (Promega). Purified RNA was reverse-transcribed and levels of *Dmpk* and *Ppib* transcripts determined by qRT-PCR with specific TaqMan assays (ThermoFisher). Log fold changes in *Dmpk* expression were calculated according to the $2^{-\Delta\Delta CT}$ method using *Ppib* as the reference gene and mice injected with vehicle as the control group. Statistical significance in differences of *Dmpk* expression between control mice and mice administered with the conjugates were determined by one-way ANOVA with Dunnet's correction for multiple comparisons. As shown in FIGs. 7B-7E, the tested conjugates showed robust activity in reducing DMPK mRNA level *in vivo* in various muscle tissues.

Example 6. Epitope Mapping

[000411] In order to determine the epitope of the hTfR1/anti-TfR Fab (3M12 VH4/Vk3) complex with high resolution, the protein complex was incubated with deuterated cross-linkers and subjected to multi-enzymatic cleavage. After enrichment of the cross-linked peptides, the samples were analyzed by high resolution mass spectrometry (nLC-LTQ-Orbitrap MS) and the data generated were analyzed using XQuest and Stavrox software.

[000412] 20 μ L of the hTfR1 (the extracellular domain of human TfR1 as set forth in SEQ ID NO: 35, amino acids C89-F760)/anti-TfR mixture prepared was mixed with 2 μ L of DSS d0/d12 (2mg/mL;DMF) before 180 minutes incubation time at room temperature. After incubation, reaction was stopped by adding 1 μ L of Ammonium Bicarbonate (20 mM final concentration) before 1 hour incubation time at room temperature. Then, the solution was dried using a speedvac before H₂O 8M urea suspension (20 μ L). After mixing, 2 μ L of DTT (500 mM) were added to the solution. The mixture was then incubated 1 hour at 37°C. After incubation, 2 μ L of iodoacetamide (1M) were added before 1 hour incubation time at room temperature, in a dark room. After incubation, 80 μ L of the proteolytic buffer were added. The trypsin buffer contains 50mM Ambic pH 8.5, 5% acetonitrile; The Chymotrypsin buffer contains Tris HCl 100mM, CaCl₂ 10mM pH 7.8; The ASP-N buffer contains Phosphate buffer

50MM pH 7.8; The elastase buffer contains Tris HCl 50mM pH 8.0 and the thermolysin buffer contains Tris HCl 50mM, CaCl₂ 0.5mM pH 9.0.

[000413] 100 µl of the reduced/alkylyed hTfR1/anti-TfR Fab mixture was mixed with 4 µl of trypsin (Promega) with the ratio 1/100. The proteolytic mixture was incubated overnight at 37°C.

[000414] 100 µl of the reduced/alkylyed hTfR1/anti-TfR Fab mixture was mixed with 2 µl of chymotrypsin (Promega) with the ratio 1/200. The proteolytic mixture was incubated overnight at 25°C.

[000415] 100 µl of the reduced/alkylyed hTfR1/anti-TfR Fab mixture was mixed with 2 µl of ASP-N (Promega) with the ratio 1/200. The proteolytic mixture was incubated overnight at 37°C.

[000416] 100 µl of the reduced/alkylyed hTfR1/anti-TfR Fab mixture was mixed with 4 µl of elastase (Promega) with the ratio 1/100. The proteolytic mixture was incubated overnight at 37°C.

[000417] 100 µl of the reduced/alkylyed hTfR1/anti-TfR Fab mixture was mixed with 8 µl of thermolysin (Promega) with a ratio 1/50. The proteolytic mixture was incubated overnight at 70°C. After digestion formic acid 1% final was added to the solution.

[000418] The samples were analyzed using nLC chromatography in combination with LTQ-Orbitrap mass spectrometry have been used. The cross-linked peptides were analyzed using Xquest version 2.0 and Stavrox 3.6. software. The nLC-orbitrap MS/MS analysis detected 15 cross-linked peptides between hTfr1 and the anti-TfR Fab. The analysis indicates that the interaction includes the following amino acids on hTfR1: K261, S273, Y282, T362, S368, S370, and K371 of SEQ ID NO: 105.

Example 7. Characterization of binding activities of anti-TfR Fab 3M12 VH4/Vk3

[000419] In vitro studies were performed to test the specificity of anti-TfR Fab 3M12 VH4/Vk3 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/Vk3 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. 8). Surface plasmon resonance (SPR) measurements were also conducted, and results are shown in Table 13. 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 13. Kinetic analysis of anti-TfR Fab 3M12 VH4/Vk3 binding to human and cynomolgus monkey TfR1 or human TfR2, measured using surface plasmon resonance

Target	Anti-TfR Fab 3M12 VH4/Vk3				
	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)

[000420] 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 μ g/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. 9).

Example 8. Serum stability of anti-TfR Fab-ASO conjugate

[000421] 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. 10 shows serum stability was similar across multiple species and remained high after 72 hours.

Example 9. Exon skipping activity of anti-TfR Fab-ASO conjugate in vivo in cynomolgus monkeys

[000422] Anti-TfR Fab 3M12 VH4/Vk3 was conjugated to a dystrophin (DMD) exon51-skipping antisense oligonucleotide (ASO) targeting an exonic splicing enhancer (ESE) sequence in DMD exon 51. The exon51 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.

Capillary electrophoresis of the PCR products was used to assess exon skipping, and % exon 51 skipping was calculated using the following formula:

$$\% \text{ Exon Skipping} = \frac{\text{Molarity of Skipped Band}}{\text{Molarity of Skipped Band} + \text{Molarity of Unskipped Band}} * 100.$$

Calculated exon 51 skipping results are shown in Table 14.

Table 14. Exon 51 skipping of dystrophin in cynomolgus monkey dystrophin

Time	2 weeks			4 weeks	
	Group	Vehicle	ASO alone ^a	Conjugate	ASO alone ^a
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)

^aASO = antisense oligonucleotide,

^bConjugate doses are listed as mg/kg of anti-TfR Fab 3M12 VH4/Vk3-ASO conjugate.

^cASO doses are listed as mg/kg ASO equivalent of the anti-TfR Fab 3M12 VH4/Vk3-ASO dose.

^dExon skipping values are mean % exon 51 skipping with standard deviations (n=5) in parentheses.

[000423] 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>gastrocnemius>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 15).

[000424] As used in this Example, the term ‘unconjugated’ indicates that the oligonucleotide was not conjugated to an antibody.

Table 15. Tissue distribution of DMD exon51 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)

^aASO = Antisense oligonucleotide.

^bConjugate doses are listed as mg/kg of anti-TfR Fab 3M12 VH4/Vk3 ASO conjugate.

^cASO doses are listed as mg/kg ASO or ASO equivalent of the anti-TfR Fab 3M12 VH4/Vk3-ASO conjugate dose.

^dASO values are mean concentrations of ASO in tissue as ng/g with standard deviations (n=5) in parentheses.

ADDITIONAL EMBODIMENTS

1. A humanized antibody that binds to human transferrin receptor (TfR), 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 humanized antibody of embodiment 1, wherein the antibody comprises 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.

3. The humanized antibody of embodiment 1, wherein the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 71 and a VL comprising the amino acid sequence of SEQ ID NO: 70.

4. The humanized antibody of embodiment 1, wherein the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 72 and a VL comprising an amino acid of SEQ ID NO: 70.
5. The humanized antibody of embodiment 1, wherein the antibody comprises 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.
6. The humanized antibody of embodiment 1, wherein the antibody comprises 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.
7. The humanized antibody of embodiment 1, wherein the antibody comprises 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.
8. The humanized antibody of embodiment 1, wherein the antibody comprises 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.
9. The humanized antibody of embodiment 1, wherein the 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.
10. The humanized antibody of embodiment 1, wherein the antibody comprises 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.
11. The humanized antibody of embodiment 1, wherein the 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: 80.
12. The humanized antibody of any one of embodiments 1-11, 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, an scFv, and an Fv.
13. The humanized antibody of embodiment 12, wherein the antibody is a full-length IgG.
14. The humanized antibody of embodiment 13, wherein the antibody comprises a heavy chain constant region of the isotype IgG1, IgG2, IgG3, or IgG4.

15. The humanized antibody of embodiment 13 or embodiment 14, 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.

16. The humanized antibody of embodiment 12, wherein the antibody is a Fab fragment.

17. The humanized antibody of embodiment 16, 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.

18. The humanized antibody of embodiment 17, wherein the antibody comprises:

(i) a heavy chain comprising the amino acid sequence of SEQ ID NO: 97; and/or 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/or 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/or 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/or 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/or 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/or 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/or 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/or 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/or 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/or a light chain comprising the amino acid sequence of SEQ ID NO: 95.

19. The humanized antibody of any one of embodiments 1 to 18, 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.

20. The humanized antibody of any one of embodiments 1-19, wherein the antibody does not specifically bind to the transferrin binding site of the transferrin receptor and/or wherein the antibody does not inhibit binding of transferrin to the transferrin receptor.
21. The humanized antibody of any one of embodiments 1-20, wherein the antibody is cross-reactive with extracellular epitopes of two or more of a human, non-human primate and rodent transferrin receptor.
22. A nucleic acid encoding the antibody in any one of embodiments 1-21.
23. A vector comprising the nucleic acid of embodiment 22.
24. A cell comprising the vector of embodiment 23.
25. A method producing an anti-TfR antibody, comprising culturing the cell of embodiment 24 under conditions suitable for the expression of the antibody.
26. A complex comprising the antibody of any one of embodiments 1-21 covalently linked to a molecular payload.
27. The complex of embodiment 26, wherein the molecular payload is a diagnostic agent or a therapeutic agent.
28. The complex of embodiment 26, wherein the molecular payload is an oligonucleotide, a polypeptide, or a small molecule.
29. The complex of any one of embodiments 26-28, wherein the antibody and the molecular payload are linked via a linker.
30. The complex of embodiment 29, wherein the linker is a cleavable linker.
31. The complex of embodiment 30, wherein the linker is comprises a valine-citrulline sequence.
32. A composition comprising the antibody of any one of embodiments 1-21, the nucleic acid of embodiment 22, the vector of embodiment 23, or the complex of any one of embodiments 26-31.
33. The composition of embodiment 32, further comprising a pharmaceutically acceptable carrier.
34. A method of detecting a transferrin receptor in a biological sample, comprising contacting the antibody of any one of embodiments 1-21 with the biological sample and measuring binding of the antibody to the biological sample.

35. The method of embodiment 34, wherein the antibody is covalently linked to a diagnostic agent.
36. The method of embodiment 35, wherein the biological sample is obtained from a human subject suspected of having or at risk for a disease associated with transferrin receptor.
37. The method of embodiment 36, wherein the contacting step is performed by administering the subject an effective amount of the anti-TfR antibody.
38. A method of delivering a molecular payload to a cell, comprising contacting the complex of any one of embodiments 26-31 with the cell.
39. The method of embodiment 38, wherein the cell is a muscle cell.
40. The method of embodiment 38 or embodiment 39, wherein the cell is in vitro.
41. The method of embodiment 40, wherein the cell is in a subject.
42. The method of embodiment 41, wherein the subject is human.
43. A method of delivering a molecular payload to the brain or the muscle of a subject, comprising administering to the subject an effective amount of the complex of any one of embodiments 26-31.
44. The method of embodiment 43, wherein the administration is intravenous.
45. A method of treating a disease, comprising administering to a subject an effective amount of the complex of any one of embodiments 26-31, wherein the molecular payload is a therapeutic agent.
46. The method of embodiment 45, wherein the disease is a neurological disease and the molecular payload is a drug for treating a neurological disease.
47. The method of embodiment 45, wherein the disease is a muscle disease and the molecular payload is a drug for treating a muscle disease.
48. The method of embodiment 47, wherein the muscle disease is a rare muscle disease or muscle atrophy.
49. An antibody that binds to human transferrin receptor (TfR), 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.

50. An antibody that binds to human transferrin receptor (TfR), wherein the antibody has undergone pyroglutamate formation resulting from a post-translational modification.

EQUIVALENTS AND TERMINOLOGY

[000425] 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.

[000426] 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.

[000427] 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.

[000428] 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.

[000429] 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.

[000430] 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. An antibody that binds to human transferrin receptor (TfR), 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 antibody 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 an amino acid sequence of SEQ ID NO: 69 and a VL comprising an amino acid sequence of SEQ ID NO: 70;
 - (iii) a VH comprising an amino acid sequence of SEQ ID NO: 71 and a VL comprising an amino acid sequence of SEQ ID NO: 70;
 - (iv) a VH comprising an amino acid sequence of SEQ ID NO: 72 and a VL comprising an amino acid sequence of SEQ ID NO: 70;
 - (v) a VH comprising an amino acid sequence of SEQ ID NO: 73 and a VL comprising an amino acid sequence of SEQ ID NO: 74;
 - (vi) a VH comprising an amino acid sequence of SEQ ID NO: 73 and a VL comprising an amino acid sequence of SEQ ID NO: 75;
 - (vii) a VH comprising an amino acid sequence of SEQ ID NO: 76 and a VL comprising an amino acid sequence of SEQ ID NO: 74;
 - (viii) a VH comprising an amino acid sequence of SEQ ID NO: 77 and a VL comprising an amino acid sequence of SEQ ID NO: 78;
 - (ix) a VH comprising an amino acid sequence of SEQ ID NO: 79 and a VL comprising an amino acid sequence of SEQ ID NO: 80; or
 - (x) a VH comprising an amino acid sequence of SEQ ID NO: 77 and a VL comprising an amino acid sequence of SEQ ID NO: 80.
3. The antibody 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, an scFv, an Fv, and a full-length IgG.
4. The antibody of claim 3, wherein the antibody is a Fab fragment.
5. The antibody 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 antibody of 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 antibody of any one of claims 1 to 6, 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.

8. The antibody of any one of claims 1 to 7, wherein the antibody does not specifically bind to the transferrin binding site of the transferrin receptor and/or wherein the antibody does not inhibit binding of transferrin to the transferrin receptor.

9. The antibody of any one of claims 1 to 8, wherein the antibody is cross-reactive with extracellular epitopes of two or more of a human, non-human primate and rodent transferrin receptor.

10. A complex comprising the antibody of any one of claims 1 to 9 covalently linked to a molecular payload.

11. The complex of claim 10, wherein the molecular payload is a diagnostic agent or a therapeutic agent.

12. The complex of claim 10, wherein the molecular payload is an oligonucleotide, a polypeptide, or a small molecule.

13. The complex of any one of claims 10 to 12, wherein the antibody and the molecular payload are linked via a linker.

14. The complex of claim 13, wherein the linker is a cleavable linker.

15. The complex of claim 13 or 14, wherein the linker comprises a valine-citrulline sequence.

16. A composition comprising the antibody of any one of claims 1 to 9 or the complex of any one of claims 10 to 15.
17. The composition of claim 16, further comprising a pharmaceutically acceptable carrier.
18. A method of delivering a molecular payload to a cell, comprising contacting the cell with the complex of any one of claims 10 to 15 or the composition of claim 16 or claim 17.
19. The method of claim 18, wherein the cell is a muscle cell.
20. The method of claim 18 or claim 19, wherein the cell is *in vitro*.
21. The method of claim 18 or claim 19, wherein the cell is in a subject.
22. The method of claim 21, wherein the subject is human.
23. A method of delivering a molecular payload to the muscle of a subject, comprising administering to the subject an effective amount of the complex of any one of claims 10 to 15 or the composition of claim 16 or claim 17.
24. The method of claim 23, wherein the administration is intravenous.
25. A method of treating a disease, comprising administering to a subject an effective amount of the complex of any one of claims 10 to 15 or the composition of claim 16 or claim 17, wherein the molecular payload is a therapeutic agent.
26. The method of claim 25, wherein the disease is a muscle disease and the molecular payload is a drug for treating the muscle disease.
27. The method of claim 26, wherein the muscle disease is a rare muscle disease or muscle atrophy.

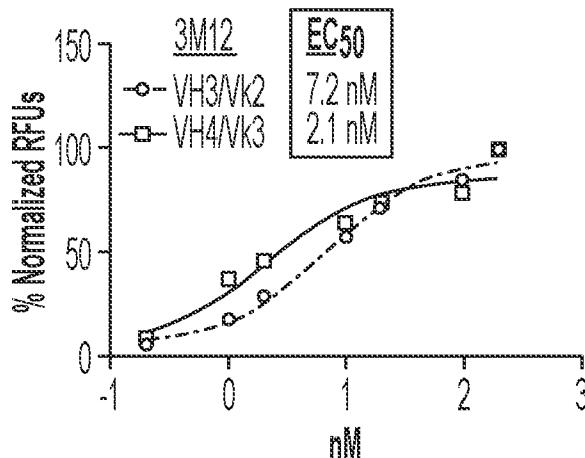


FIG. 1A

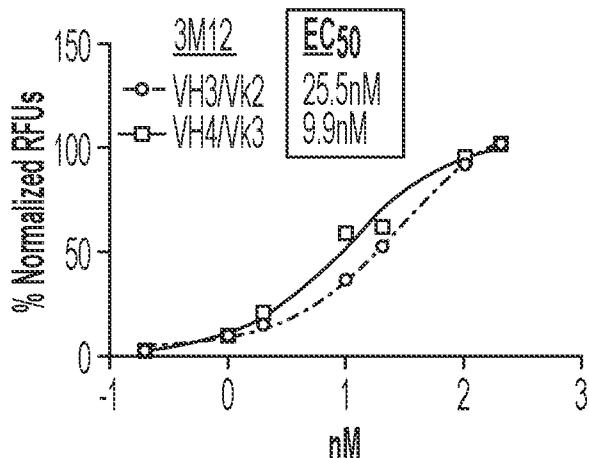


FIG. 1B

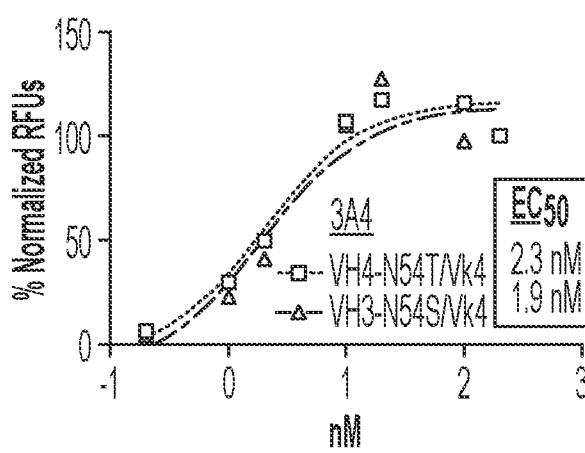


FIG. 1C

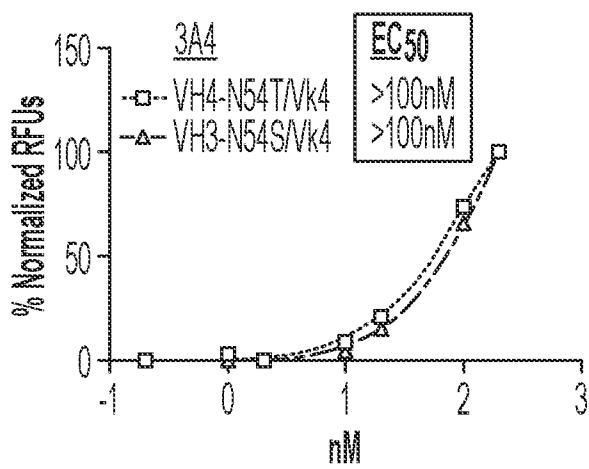


FIG. 1D

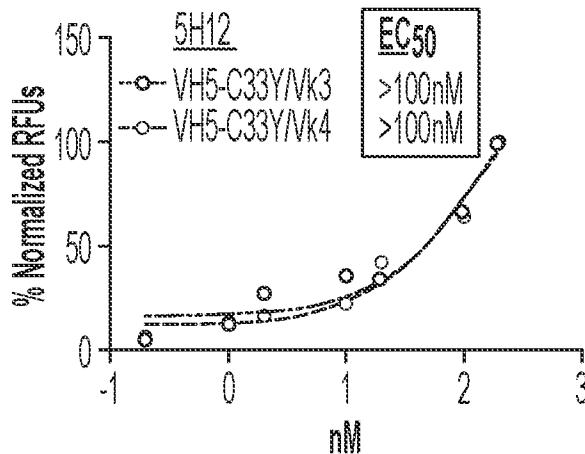


FIG. 1E

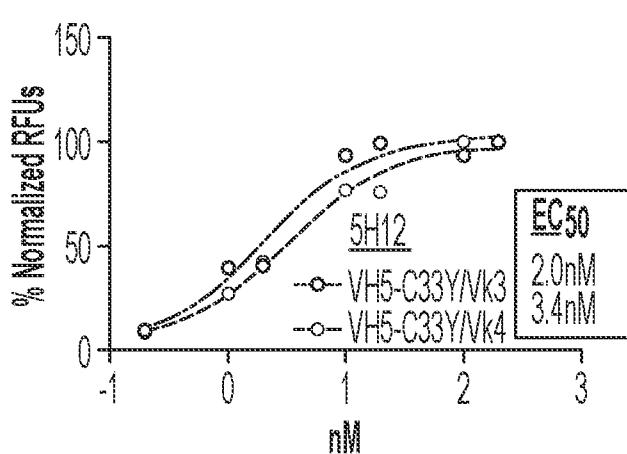


FIG. 1F

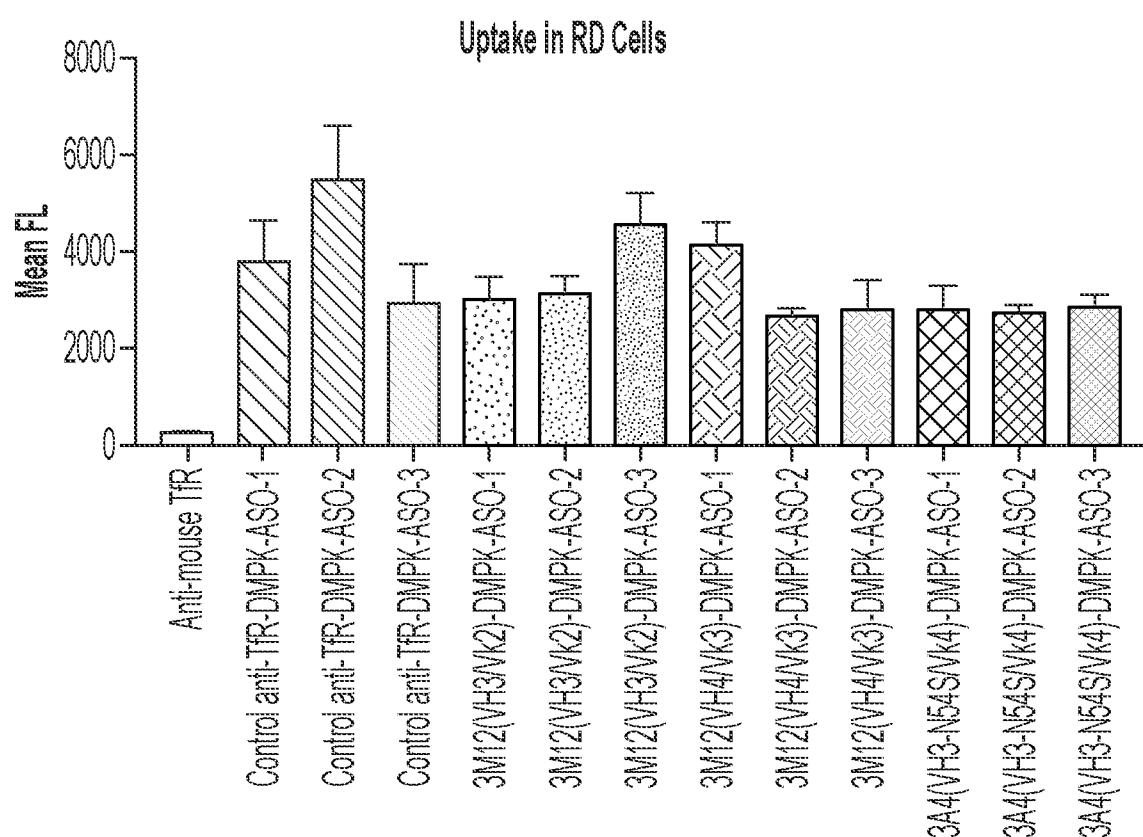


FIG. 2

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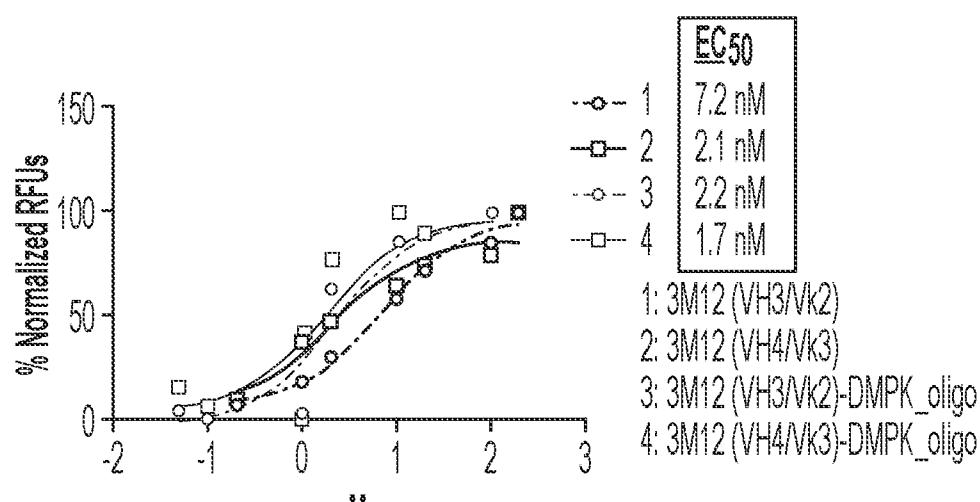


FIG. 3A

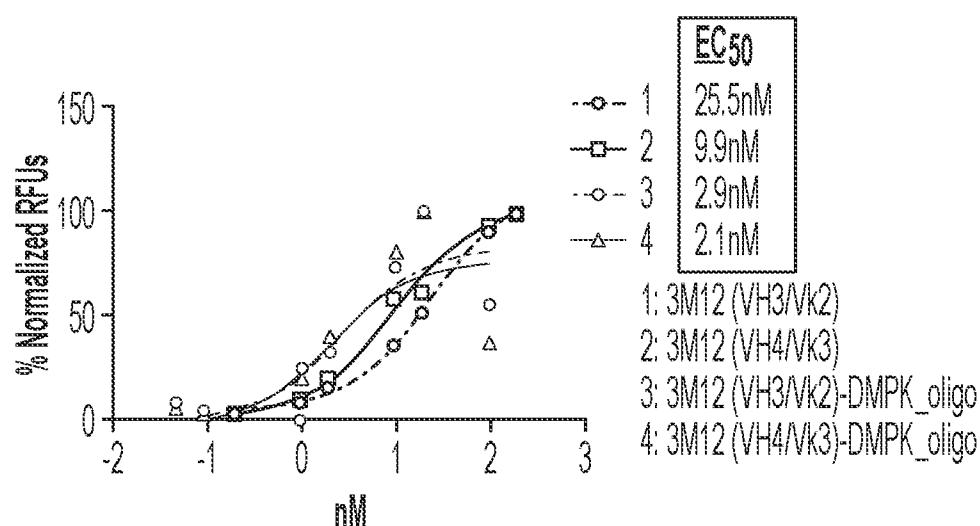


FIG. 3B

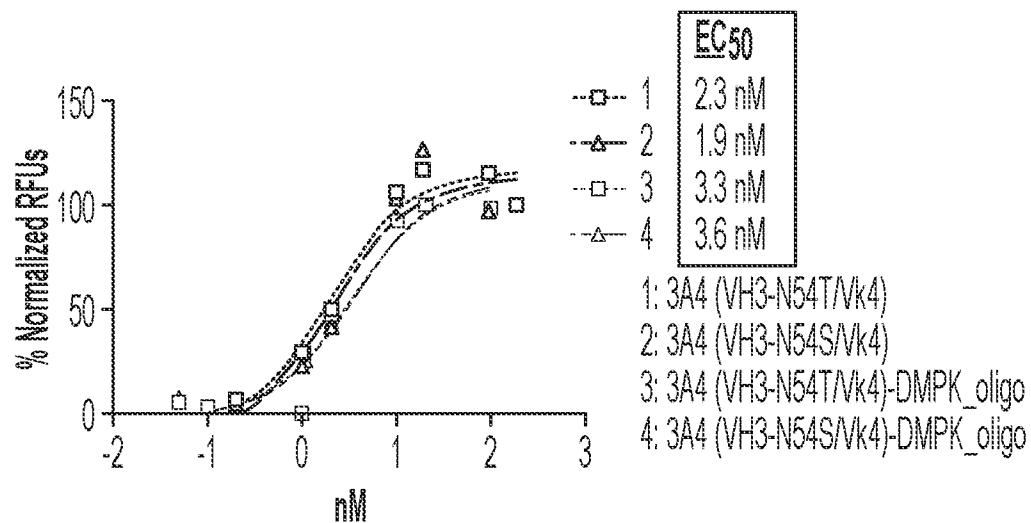


FIG. 3C

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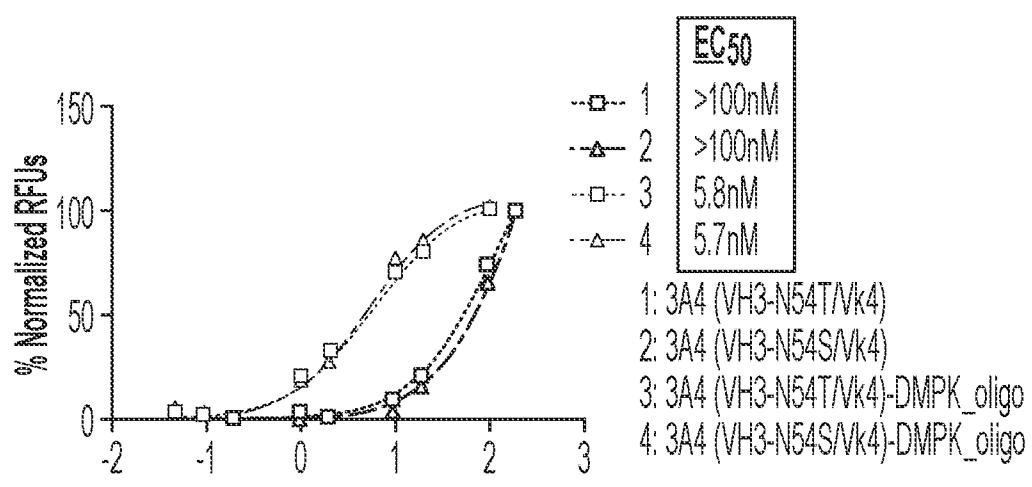


FIG. 3D

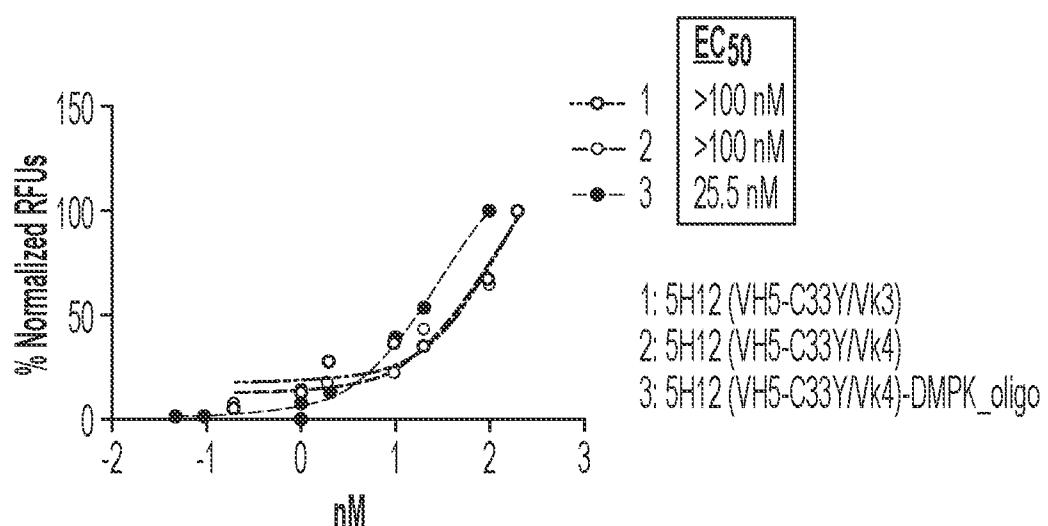


FIG. 3E

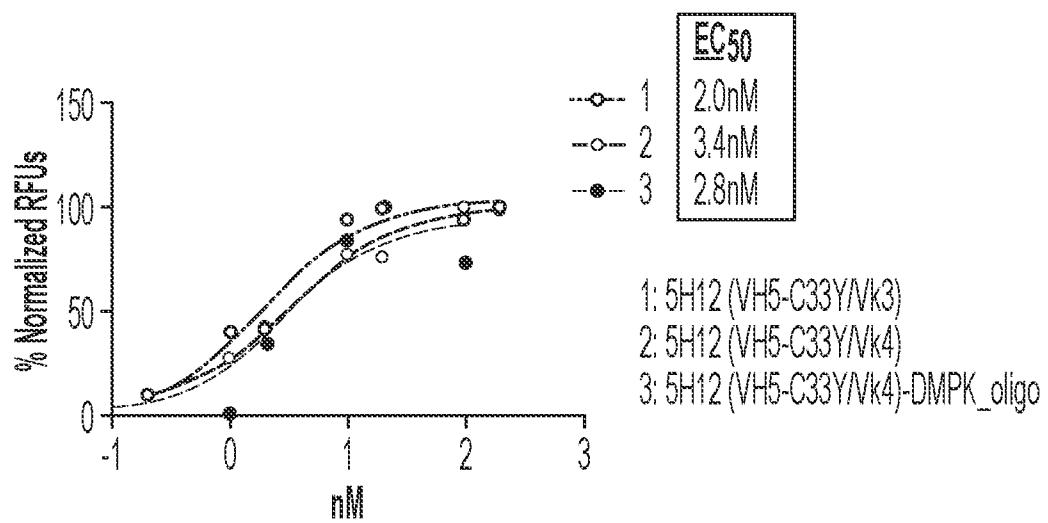


FIG. 3F

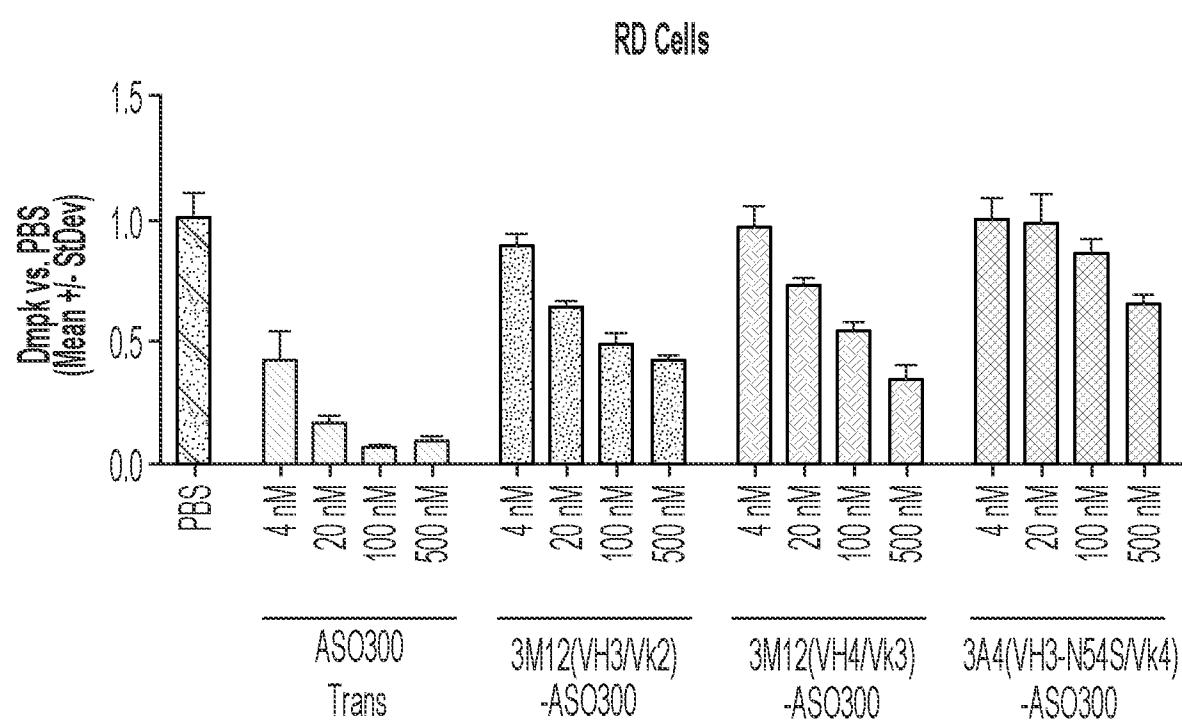
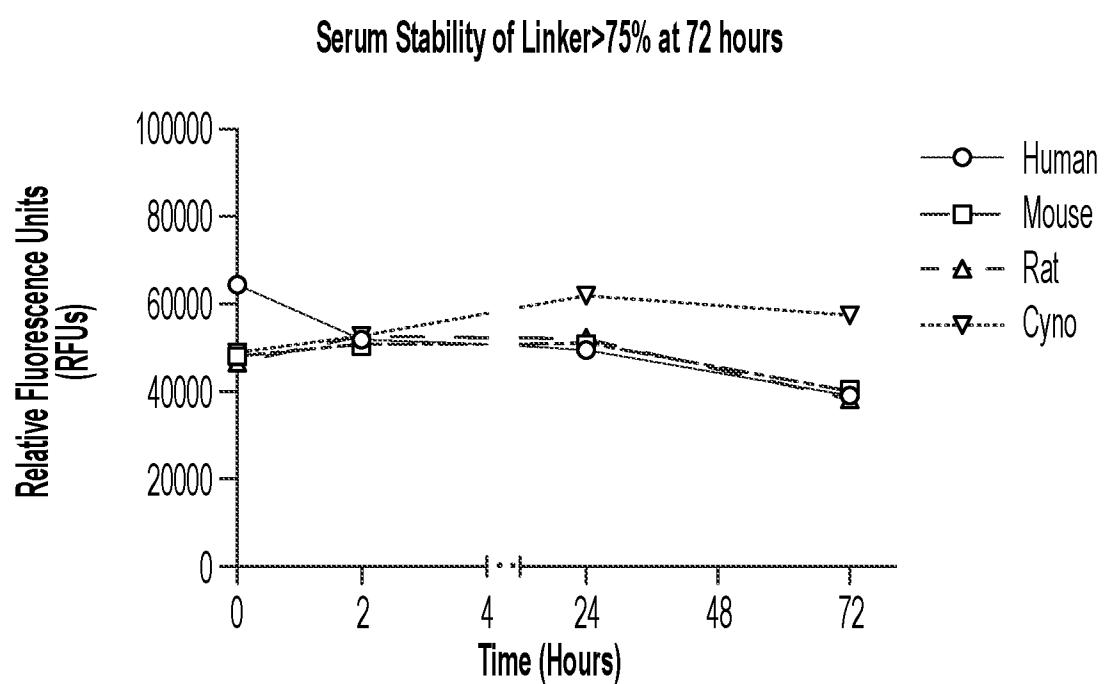
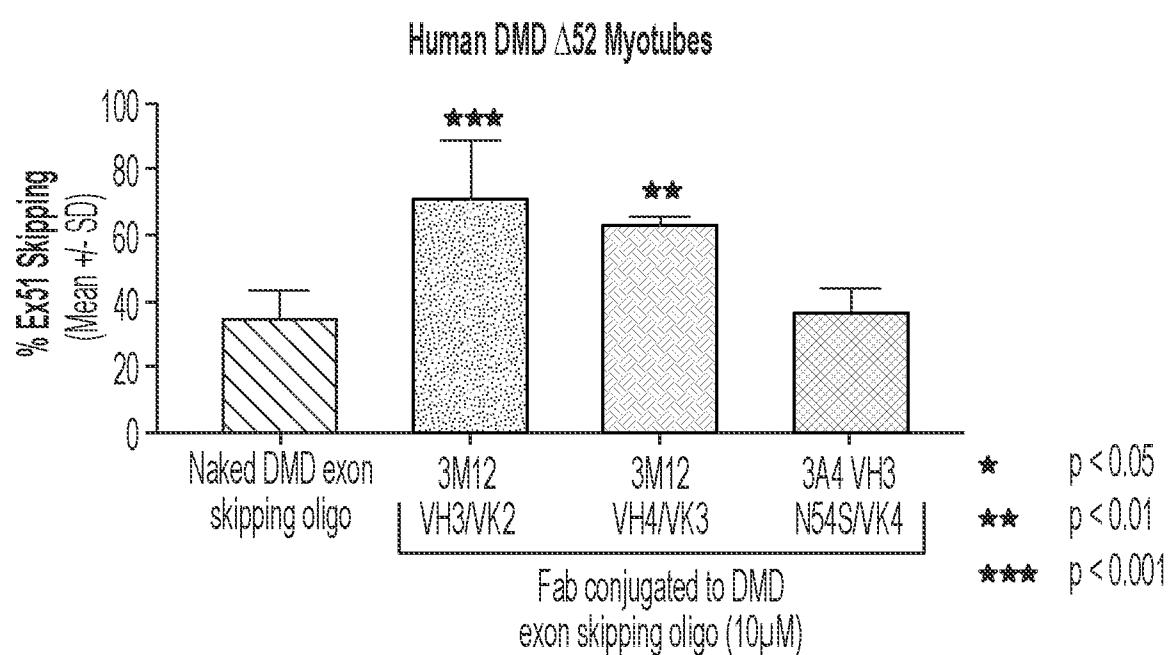


FIG. 4

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**FIG. 5**

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**FIG. 6**

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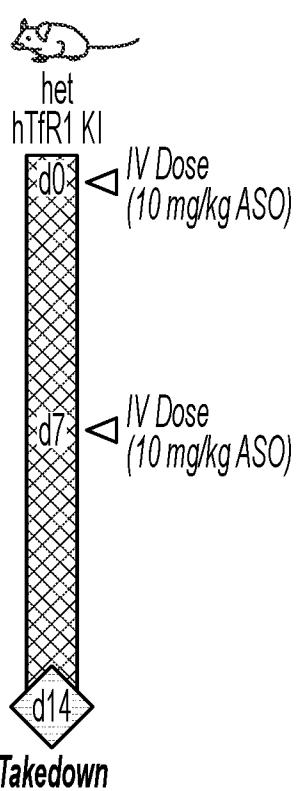


FIG. 7A

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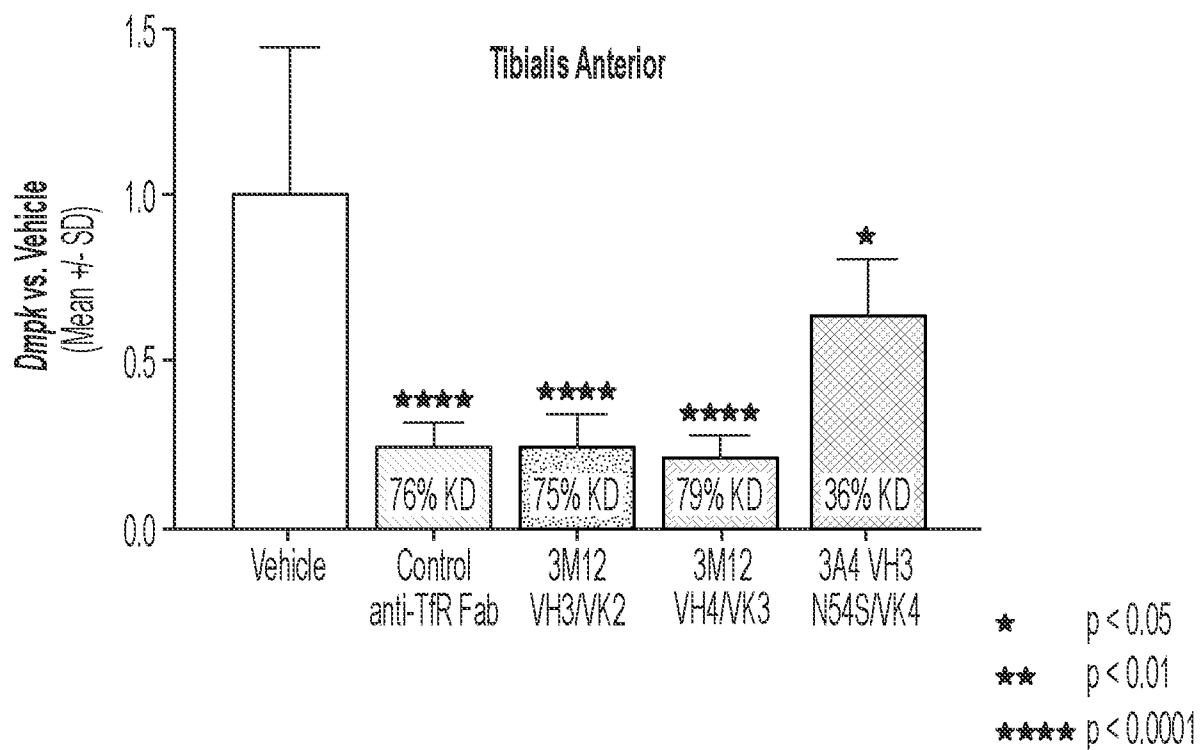


FIG. 7B

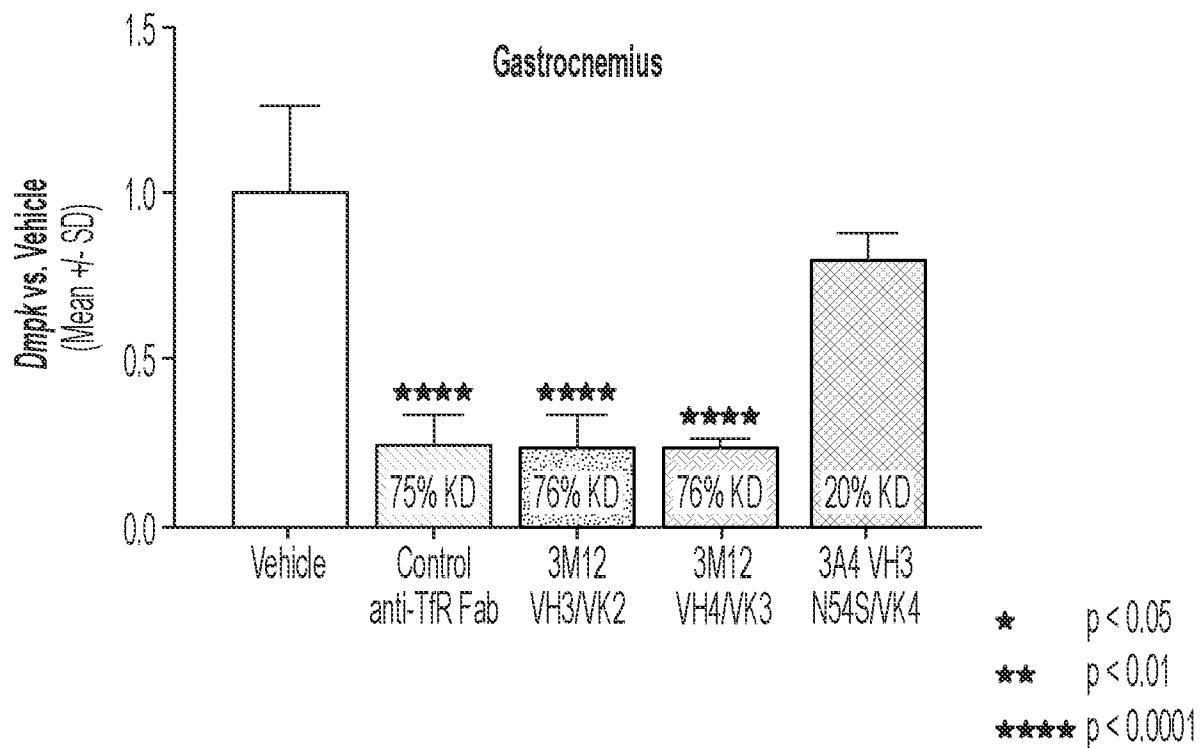


FIG. 7C

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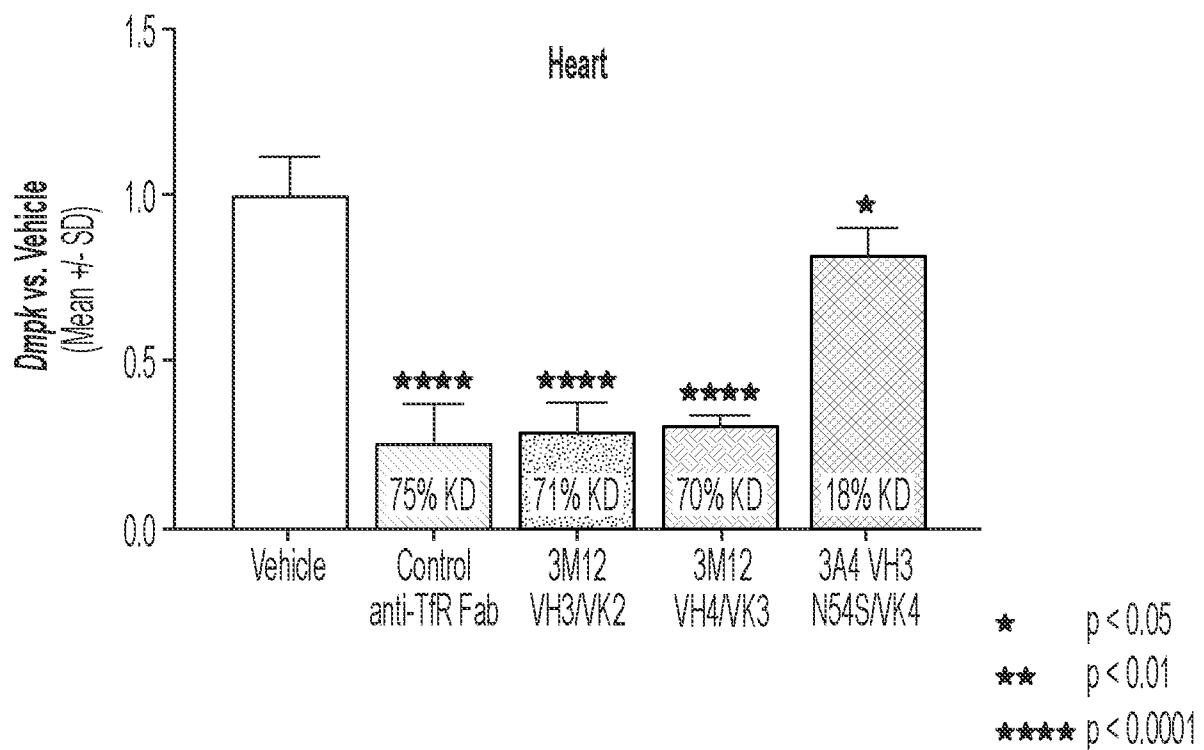


FIG. 7D

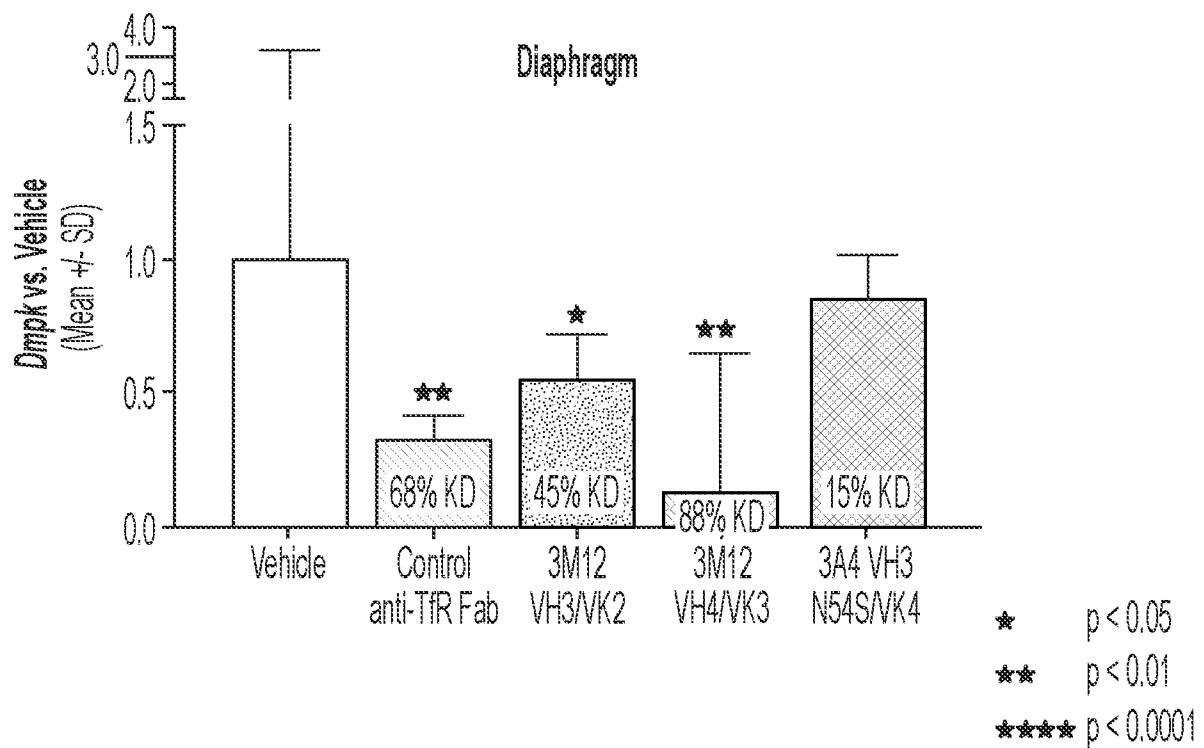


FIG. 7E

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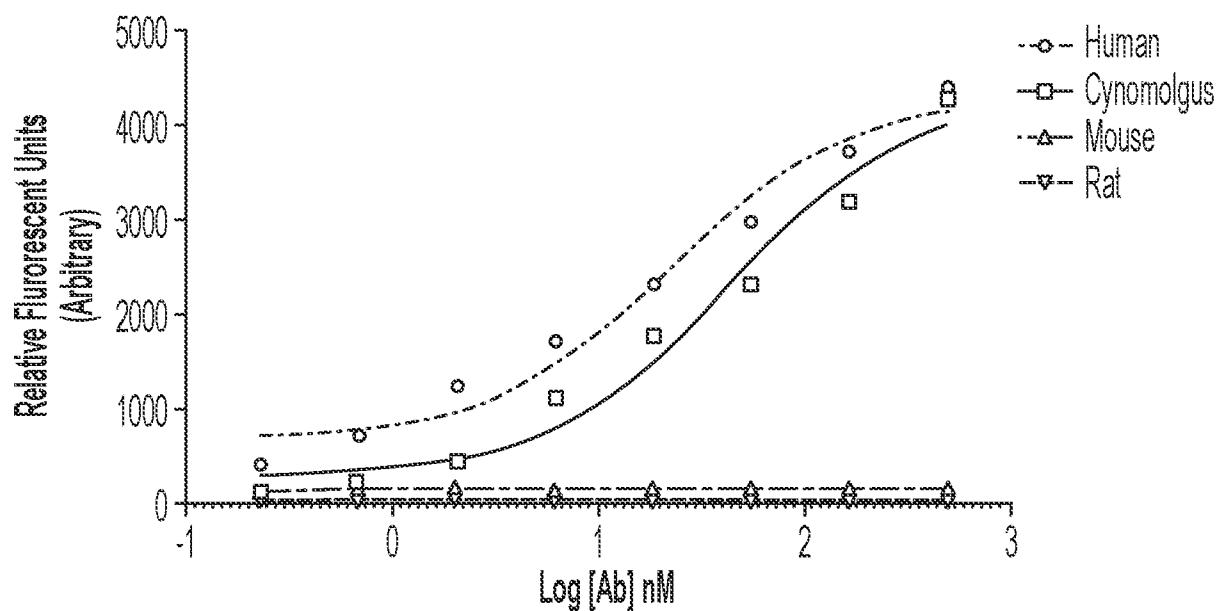


FIG. 8

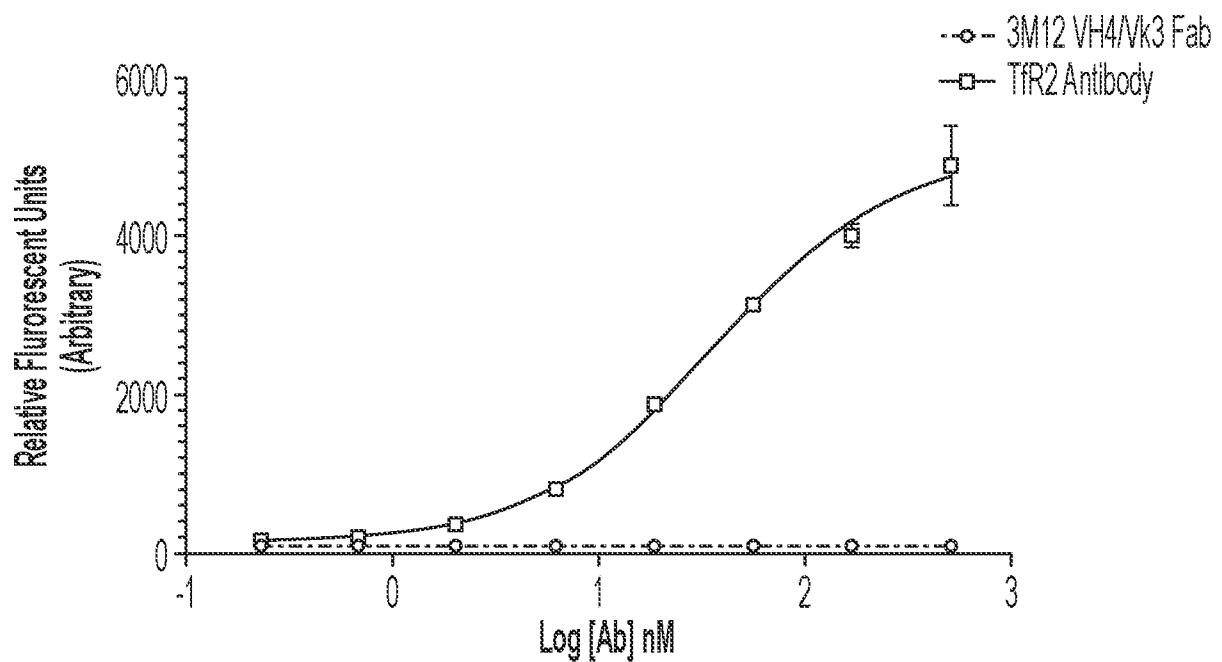


FIG. 9

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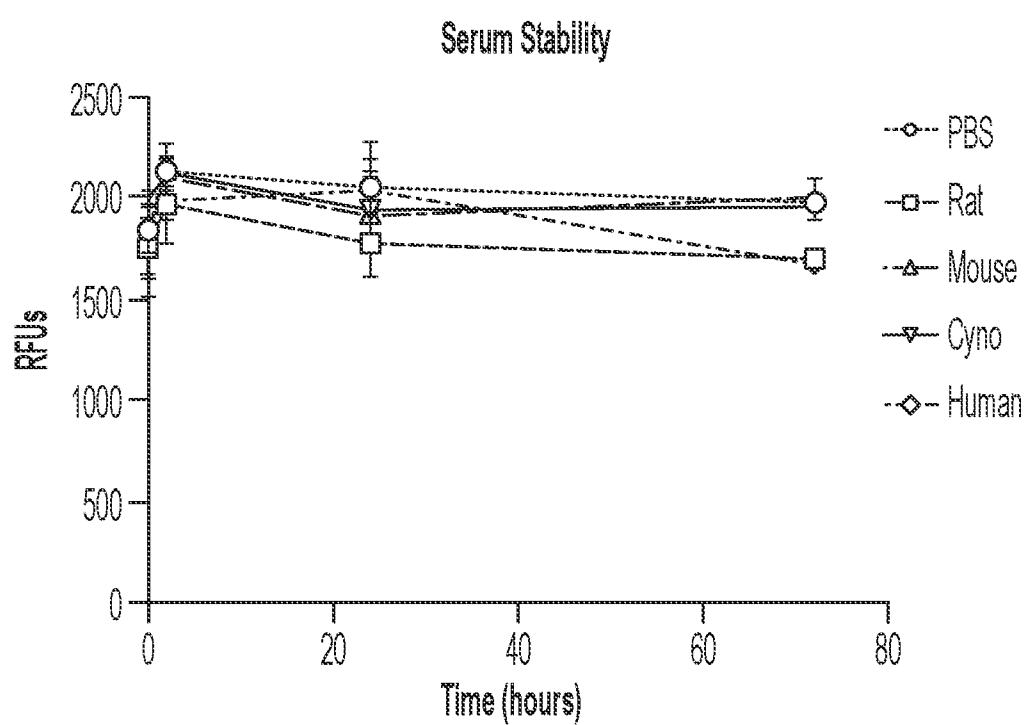


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/040984

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 38/02; A61K 39/395; A61K 47/68 (2021.01)
 CPC - A61K 38/02; A61K 39/395; A61K 47/68 (2021.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 see Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 see Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 see Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2013/0028891 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 31 January 2013 (31.01.2013) entire document	1-4
A	WO 2019/229658 A1 (NOVARTIS AG) 05 December 2019 (05.12.2019) entire document	1-4
A	US 2019/0153083 A1 (UNIVERSITE FRANCOIS RABELAIS) 23 May 2019 (23.05.2019) entire document	1-4

Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 21 November 2021	Date of mailing of the international search report DEC 27 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Harry Kim Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/040984

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 7-27 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see extra sheet(s).

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-4

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/040984

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. forming part of the international application as filed:
 - in the form of an Annex C/ST.25 text file.
 - on paper or in the form of an image file.
 - b. furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
 - c. furnished subsequent to the international filing date for the purposes of international search only:
 - in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
 - on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).
2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 69-80, 90, and 101 were searched.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/040984

Continued from Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-6 are drawn to antibodies that bind to human transferrin receptor (TfR).

The first invention of Group I+ is restricted to an antibody that binds to human transferrin receptor (TfR), wherein the antibody comprises: (i) a heavy chain variable region (VH), and a light chain variable region (VL), where the VH is selected to be SEQ ID NO:76, and the VL is selected to be SEQ ID NO:75. It is believed that claims 1-4 read on this first named invention and thus these claims will be searched without fee to the extent that they read on SEQ ID NOs: 75 and 76.

Applicant is invited to elect additional VH and VL, each with specified SEQ ID NO to be searched in a specific combination by paying additional fee for each set of election. An exemplary election would be an antibody that binds to human transferrin receptor (TfR), wherein the antibody comprises: (i) a heavy chain variable region (VH), and a light chain variable region (VL), where the VH is selected to be SEQ ID NO:69, and the VL is selected to be SEQ ID NO:70. Additional VH and VL, each with specified SEQ ID NO will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulas do not share a significant structural element responsible for binding to human transferrin receptors, requiring the selection of alternative VH and VL where "An antibody that binds to human transferrin receptor (TfR), 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."

Additionally, even if Groups I+ were considered to share the technical features of an antibody that binds to human transferrin receptor (TfR), wherein the antibody comprises: (i) a heavy chain variable region (VH), and a light chain variable region (VL). However, these shared technical features do not represent a contribution over the prior art.

Specifically, US 2013/0028891 A1 to The Regents Of The University Of California discloses an antibody that binds to human transferrin receptor (TfR) (anti-TfR antibody is reactive with at least a portion of a human transferrin receptor, Para. [0008]), wherein the antibody comprises: (i) a heavy chain variable region (VH), and a light chain variable region (VL) (anti-TfR antibody comprises light and heavy chain variable regions, Para. [0008]).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical features.