



(12) **DEMANDE DE BREVET CANADIEN**  
**CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2019/08/02  
(87) Date publication PCT/PCT Publication Date: 2020/02/06  
(85) Entrée phase nationale/National Entry: 2021/02/01  
(86) N° demande PCT/PCT Application No.: US 2019/044959  
(87) N° publication PCT/PCT Publication No.: 2020/028840  
(30) Priorité/Priority: 2018/08/02 (US62/714,035)

(51) Cl.Int./Int.Cl. *C07K 16/28* (2006.01),  
*A61K 39/395* (2006.01), *C12N 15/113* (2010.01)  
(71) Demandeur/Applicant:  
DYNE THERAPEUTICS, INC., US  
(72) Inventeurs/Inventors:  
SUBRAMANIAN, ROMESH R., US;  
QATANANI, MOHAMMED T., US;  
WEEDEN, TIMOTHY, US  
(74) Agent: RIDOUT & MAYBEE LLP

(54) Titre : COMPLEXES DE CIBLAGE MUSCULAIRE ET LEURS UTILISATIONS POUR LE TRAITEMENT DE L'ATAXIE DE FRIEDREICH

(54) Title: MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING FRIEDREICH'S ATAXIA

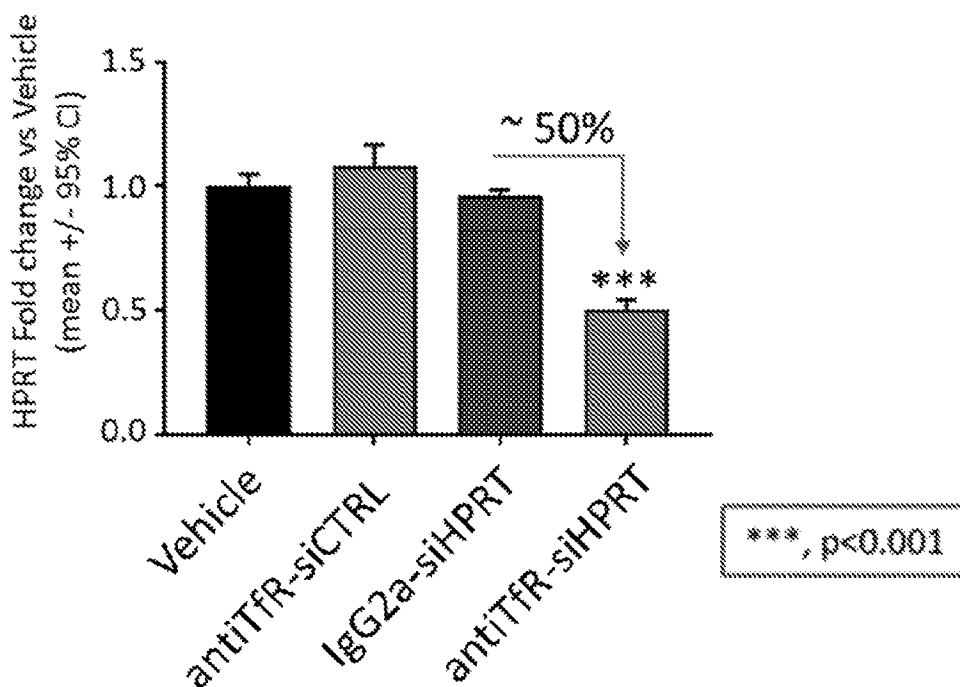


FIG. 2

(57) Abrégé/Abstract:

Aspects of the disclosure relate to complexes comprising a muscle-targeting agent covalently linked to a molecular payload. In some embodiments, the muscle-targeting agent specifically binds to an internalizing cell surface receptor on muscle cells. In some

(57) **Abrégé(suite)/Abstract(continued):**

embodiments, the molecular payload increases FXN expression. In some embodiments, the molecular payload is an oligonucleotide, such as an antisense oligonucleotide or RNAi oligonucleotide.

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

06 February 2020 (06.02.2020)



(10) International Publication Number

WO 2020/028840 A1

## (51) International Patent Classification:

C07K 16/28 (2006.01) C12N 15/113 (2010.01)

A61K 39/395 (2006.01)

## (21) International Application Number:

PCT/US2019/044959

## (22) International Filing Date:

02 August 2019 (02.08.2019)

## (25) Filing Language:

English

## (26) Publication Language:

English

## (30) Priority Data:

62/714,035 02 August 2018 (02.08.2018) US

(71) Applicant: **DYNE THERAPEUTICS, INC.** [US/US];  
400 Technology Square, 10th Floor, Cambridge, MA 02139 (US).

(72) Inventors: **SUBRAMANIAN, Romesh, R.**; 400 Technology Square, 10th Floor, Cambridge, MA 02139 (US).  
**QATANANI, Mohammed, T.**; 400 Technology Square, 10th Floor, Cambridge, MA 02139 (US).  
**WEEDEN, Timothy**; 400 Technology Square, 10th Floor, Cambridge, MA 02139 (US).

(74) Agent: **YOUNG, Daniel, W.** et al.; Wolf, Greenfield & Sacks, P.C, 600 Atlantic Avenue, Boston, MA 02210-2206 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING FRIEDREICH'S ATAXIA

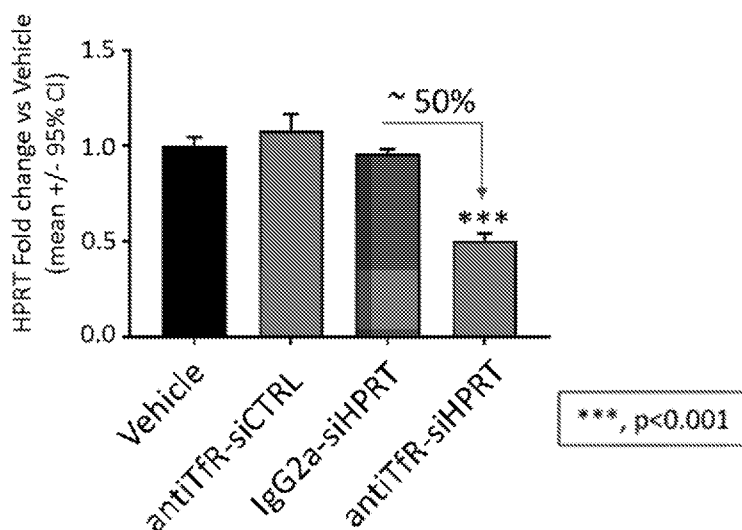


FIG. 2

(57) Abstract: Aspects of the disclosure relate to complexes comprising a muscle-targeting agent covalently linked to a molecular payload. In some embodiments, the muscle-targeting agent specifically binds to an internalizing cell surface receptor on muscle cells. In some embodiments, the molecular payload increases FXN expression. In some embodiments, the molecular payload is an oligonucleotide, such as an antisense oligonucleotide or RNAi oligonucleotide.

[Continued on next page]

# WO 2020/028840 A1

---

**Published:**

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

- 1 -

## **MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING FRIEDREICH'S ATAXIA**

### **RELATED APPLICATIONS**

**[0001]** This application claims the benefit of the filing date of U.S. Provisional Application No. 62/714,035, entitled "MUSCLE TARGETING COMPLEXES AND USES THEREOF FOR TREATING FRIEDREICH'S ATAXIA", filed August 2, 2018; the contents of which is incorporated herein by reference in its entirety.

### **FIELD OF THE INVENTION**

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

### **REFERENCE TO THE SEQUENCE LISTING**

**[0003]** The present application is being filed along with a Sequence Listing in electronic format. The Sequence Listing is provided as a file entitled D082470005WO00-SEQ.txt created on July 31, 2019 which is 56 kilobytes in size. The information in electronic format of the sequence listing is incorporated herein by reference in its entirety.

### **BACKGROUND OF INVENTION**

**[0004]** Friedreich's ataxia is a rare, autosomal recessive disease that leads to progressive damage of muscle tissues and the nervous system. Hallmarks of the disease include severe heart conditions, *e.g.* hypertrophic cardiomyopathy, myocardial fibrosis, and heart failure, and degeneration of nerve fibers in the spinal cord and peripheral nervous system. Friedreich's ataxia results from a mutation in the FXN gene, which codes for frataxin, a protein proposed to function in iron homeostasis. Specifically, subjects with the disease have an expanded trinucleotide GAA sequence that leads to decreased levels of frataxin. Friedreich's ataxia is the most common form of hereditary ataxia, with an incidence of about 1 in every 50,000 people. In the most severe cases, subjects with the disease may be unable to walk freely by the age of 10-20 and many subjects experience shortened life expectancy. In Friedreich's ataxia, the reduction of FXN expression may be attributable to epigenetic silencing and/or from a decreased ability to splice out the first intron of FXN pre-mRNA, which contains an expanded GAA repeat. With

- 2 -

the exception of supportive care to combat symptoms of the disease, there are no current and effective treatments available for Friedreich's ataxia.

## SUMMARY OF INVENTION

**[0005]** According to some aspects, the disclosure provides complexes that target muscle cells for purposes of delivering molecular payloads to those cells. In some embodiments, complexes provided herein are particularly useful for delivering molecular payloads that increase FXN expression, *e.g.*, in a subject having or suspected of having Friedreich's ataxia. Accordingly, in some embodiments, complexes provided herein comprise muscle-targeting agents (*e.g.*, muscle targeting antibodies) that specifically bind to receptors on the surface of muscle cells for purposes of delivering molecular payloads to the muscle cells. In some embodiments, the complexes are taken up into the cells via a receptor mediated internalization, following which the molecular payload may be released to perform a function inside the cells. For example, complexes engineered to deliver oligonucleotides may release the oligonucleotides such that the oligonucleotides can increase FXN gene expression in the muscle cells. In some embodiments, the oligonucleotides are released by endosomal cleavage of covalent linkers connecting oligonucleotides and muscle-targeting agents of the complexes.

**[0006]** Some aspects of the disclosure comprise a complex comprising a muscle-targeting agent covalently linked to a molecular payload configured for increasing FXN expression, wherein the muscle-targeting agent specifically binds to an internalizing cell surface receptor on muscle cells, wherein the muscle-targeting agent specifically binds to an internalizing cell surface receptor on muscle cells.

**[0007]** In some embodiments, the muscle-targeting agent is a muscle-targeting antibody. In some embodiments, a muscle-targeting antibody is an antibody that specifically binds to an extracellular epitope of a transferrin receptor (*e.g.*, an epitope of the apical domain of the transferrin receptor). A muscle-targeting antibody may specifically binds to an epitope of a sequence in the range of C89 to F760 of SEQ ID NO: 1-3. In some embodiments, the equilibrium dissociation constant (K<sub>d</sub>) of binding of a muscle-targeting antibody to a transferrin receptor is in a range from 10<sup>-11</sup> M to 10<sup>-6</sup> M.

**[0008]** In some embodiments, a muscle-targeting antibody of a complex competes for specific binding to an epitope of a transferrin receptor with an antibody listed in Table 1 (*e.g.*,

- 3 -

competes for specific binding to an epitope of a transferrin receptor with an  $K_d$  of less than or equal to  $10^{-6}$  M, *e.g.*, in a range of  $10^{-11}$  M to  $10^{-6}$  M).

**[0009]** In some embodiments, a muscle-targeting antibody of a complex does not specifically bind to the transferrin binding site of a transferrin receptor and/or does not inhibit binding of transferrin to a transferrin receptor. In some embodiments, a muscle-targeting antibody of a complex is cross-reactive with extracellular epitopes of two or more of a human, non-human primate and rodent transferrin receptor. In some embodiments, a muscle-targeting antibody of a complex is configured to promote transferrin receptor mediated internalization of the molecular payload into a muscle cell.

**[0010]** A muscle-targeting antibody (*e.g.*, muscle-targeting antibody is an antibody that specifically binds to an extracellular epitope of a transferrin receptor) is a chimeric antibody, wherein optionally the chimeric antibody is a humanized monoclonal antibody. A muscle-targeting antibody may be in the form of a ScFv, Fab fragment, Fab' fragment, F(ab')<sub>2</sub> fragment, or Fv fragment.

**[0011]** In some embodiments, a molecular payload of a complex is an oligonucleotide (*e.g.*, comprising DNA and/or RNA). In some embodiments, an oligonucleotide comprises a region of complementarity to an FXN allele. In some embodiments, an oligonucleotide comprises an antisense strand having a region of complementarity to a GAA trinucleotide sequence. In some embodiments, an oligonucleotide inhibits R-loop formation in a FXN allele.

**[0012]** An oligonucleotide of the disclosure may comprise at least one modified internucleotide linkage (*e.g.*, a phosphorothioate linkage). In some embodiments, an oligonucleotide comprises phosphorothioate linkages in the Rp stereochemical conformation and in the Sp stereochemical conformation. In some embodiments, an oligonucleotide comprises phosphorothioate linkages that are all in the Rp stereochemical conformation. In other embodiments, an oligonucleotide comprises phosphorothioate linkages that are all in the Sp stereochemical conformation.

**[0013]** An oligonucleotide of the disclosure may comprise one or more modified nucleotides (*e.g.*, 2'-modified nucleotides). In some embodiments, a modified nucleotide is a 2'-O-methyl, 2'-fluoro (2'-F), 2'-O-methoxyethyl (2'-MOE), or 2', 4'-bridged nucleotide. In some embodiments, a modified nucleotides is a bridged nucleotide (*e.g.*, selected from: 2',4'-constrained 2'-O-ethyl (cEt) and locked nucleic acid (LNA) nucleotides).

- 4 -

**[00014]** In some embodiments, an oligonucleotide is a gapmer oligonucleotide. A gapmer oligonucleotide may comprise a central portion of 5 to 15 deoxyribonucleotides flanked by wings of 2 to 8 modified nucleotides (*e.g.*, 2'-modified nucleotides).

**[00015]** In some embodiments, an oligonucleotide is a mixmer oligonucleotide. A mixmer oligonucleotide may comprise two or more different 2' modified nucleotides.

**[00016]** In some embodiments, an oligonucleotide is an RNAi oligonucleotide. An RNAi oligonucleotide may be a double-stranded oligonucleotide of 19 to 25 nucleotides in length. In some embodiments, an RNAi oligonucleotide comprises at least one 2' modified nucleotide.

**[00017]** In some embodiments, an oligonucleotide comprises a guide sequence for a genome editing nuclease.

**[00018]** In some embodiments, an oligonucleotide is a phosphorodiamidite morpholino oligomer (PMO).

**[00019]** In some embodiments, an oligonucleotide is an mRNA encoding frataxin, a fragment of frataxin, or a variant of frataxin. In some embodiments, an mRNA comprises a 5' cap structure and/or a 3' polyA tail.

**[00020]** In other embodiments, a molecular payload is a polypeptide. In some embodiments, a molecular payload is a polypeptide that comprises frataxin, a fragment of frataxin, or a variant of frataxin.

**[00021]** In some embodiments, a muscle-targeting agent is covalently linked to a molecular payload via a cleavable linker (*e.g.*, a protease-sensitive linker, pH-sensitive linker, or glutathione-sensitive linker). A protease-sensitive linker may comprise a sequence cleavable by a lysosomal protease and/or an endosomal protease. In some embodiments, a protease-sensitive linker comprises a valine-citrulline dipeptide sequence. A pH-sensitive linker may be cleaved at a pH in a range of 4 to 6.

**[00022]** In some embodiments, a muscle-targeting agent is covalently linked to a molecular payload via a non-cleavable linker (*e.g.*, an alkane linker).

**[00023]** In some embodiments, a muscle-targeting antibody comprises a non-natural amino acid to which an oligonucleotide can be covalently linked. In some embodiments, a muscle-targeting antibody is covalently linked to an oligonucleotide via conjugation to a lysine

- 5 -

residue or a cysteine residue of the antibody. In some embodiments, an oligonucleotide is conjugated to a cysteine residue of the antibody via a maleimide-containing linker, optionally wherein the maleimide-containing linker comprises a maleimidocaproyl or maleimidomethyl cyclohexane-1-carboxylate group.

**[00024]** In some embodiments, a muscle-targeting antibody is a glycosylated antibody that comprises at least one sugar moiety to which a oligonucleotide is covalently linked. In some embodiments, a glycosylated antibody that comprises at least one sugar moiety that is a branched mannose. In some embodiments, a muscle-targeting antibody is a glycosylated antibody that comprises one to four sugar moieties each of which is covalently linked to a separate oligonucleotide. In some embodiments, a muscle-targeting antibody is a fully-glycosylated antibody or a partially-glycosylated antibody. A partially-glycosylated antibody may be produced via chemical or enzymatic means. In some embodiments, a partially-glycosylated antibody is produced in a cell that is deficient for an enzyme in the N- or O-glycosylation pathway.

**[00025]** Some aspects of the disclosure comprise a method of delivering an molecular payload to a cell expressing transferrin receptor, the method comprising contacting the cell with a complex comprising a muscle-targeting agent covalently linked to a molecular payload configured for increasing FXN expression.

**[00026]** Some aspects of the disclosure comprise a method of increasing expression of FXN in a cell, the method comprising contacting the cell with a complex comprising a muscle-targeting agent covalently linked to a molecular payload configured for increasing FXN expression in an amount effective for promoting internalization of the molecular payload to the cell. In some embodiments, the cell is *in vitro*. In some embodiments, the cell is in a subject. In some embodiments, the subject is a human.

**[00027]** Some aspects of the disclosure comprise a method of treating a subject having a mutant FXN allele that is associated with Friedreich's ataxia, the method comprising administering to the subject an effective amount of a complex comprising a muscle-targeting agent covalently linked to a molecular payload configured for increasing FXN expression.

#### BRIEF DESCRIPTION OF THE DRAWINGS

- 6 -

[00028] FIG. 1 depicts a non-limiting schematic showing the effect of transfecting cells with an siRNA.

[00029] FIG. 2 depicts a non-limiting schematic showing the activity of a muscle targeting complex comprising an siRNA.

[00030] FIGs. 3A-3B depict non-limiting schematics showing the activity of a muscle targeting complex comprising an siRNA in mouse muscle tissues (gastrocnemius and heart) *in vivo*, relative to control experiments. (N=4 C57BL/6 WT mice)

[00031] FIGs. 4A-4E depict non-limiting schematics showing the tissue selectivity of a muscle targeting complex comprising an siRNA.

#### DETAILED DESCRIPTION OF INVENTION

[00032] Aspects of the disclosure relate to a recognition that while certain molecular payloads (*e.g.*, oligonucleotides, peptides, small molecules) can have beneficial effects in muscle cells, it has proven challenging to effectively target such cells. As described herein, the present disclosure provides complexes comprising muscle-targeting agents covalently linked to molecular payloads in order to overcome such challenges. In some embodiments, complexes are provided for increasing FXN expression to treat subjects having Friedreich's ataxia. For example, in some embodiments, complexes provided herein may comprise oligonucleotides that increase expression of FXN. As one example, oligonucleotides may target an R-loop portion of FXN that has an expansion of GAA repeats in order to increase frataxin expression. In some embodiments, inhibition of R-loop formation, *e.g.* with a molecular payload capable of binding to the expanded GAA repeat, can allow for normal expression of the FXN gene and treatment of the disease. However, in some embodiments, complexes provided herein may comprise synthetic nucleic acid payloads (*e.g.*, DNA or RNA payloads) engineered to express FXN. In some embodiments, complexes provided herein may comprise molecular payloads such as guide molecules (*e.g.*, guide RNAs) that are capable of targeting nucleic acid programmable nucleases (*e.g.*, Cas9) to a sequence at or near a disease-associated repeat GAA sequence of FXN or comprise nucleases (*e.g.* Cas9, TALENs, etc.) that can edit DNA or RNA. In some embodiments, such nucleic programmable nucleases could be used to cleave part or all of a disease-associated repeat sequence from a FXN allele.

[00033] Further aspects of the disclosure, including a description of defined terms, are provided below.

## I. Definitions

**[00034] 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 pharmacologically useful (*e.g.*, to treat a condition in the subject).

**[00035] 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).

**[00036] 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 F(ab')<sub>2</sub> 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 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

- 8 -

particular embodiment, an antibody described herein comprises a human gamma 1 CH1, CH2, and/or CH3 domain. In some embodiments, the amino acid sequence of the VH domain comprises the amino acid sequence of a human gamma ( $\gamma$ ) 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 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 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).

- 9 -

**[00037] CDR:** As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. 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, although preferred embodiments use Kabat or Chothia defined CDRs.

**[00038] 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 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.

**[00039] 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.

- 10 -

**[00040] 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.

**[00041] 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.

**[00042] 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.

- 11 -

**[00043] 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 transferring 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.

**[00044] Disease-associated-repeat:** As used herein, the term “disease-associated-repeat” refers to a repeated nucleotide sequence at a genomic location for which the number of units of the repeated nucleotide sequence is correlated with and/or directly or indirectly contributes to, or causes, genetic disease. In some embodiments, a disease associated repeat is a trinucleotide repeat. In some embodiments, the disease-associated-repeat comprises GAA repeats or a nucleotide complement of any thereof. In some embodiments, a disease-associated-repeat is in a non-coding portion of a gene. In some embodiments, a disease-associated-repeat is expanded from a normal state to a length that directly or indirectly contributes to, or causes, genetic disease. In some embodiments, a disease-associated-repeat is in RNA (e.g., an RNA transcript). In some embodiments, a disease-associated-repeat is in DNA (e.g., a chromosome, a plasmid). In some embodiments, a disease-associated-repeat is expanded in a chromosome of a germline cell. In some embodiments, a disease-associated-repeat is expanded in a chromosome of a somatic cell. In some embodiments, a disease-associated-repeat is expanded to a number of repeating units that is associated with onset of disease.

**[00045] 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-

- 12 -

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.

**[00046]      Friedrich's ataxia:** As used herein, the term "Friedreich's ataxia" refers to an autosomal recessive genetic disease caused by mutations in the FXN gene and is characterized by progressive damage of muscle tissues and the nervous system. Friedreich's ataxia is associated with an expansion of a GAA trinucleotide repeat in the FXN gene that leads to a decrease in the expression of FXN. The expanded GAA trinucleotide repeat, located within the first intron, forms a R-loop which can interfere with normal transcriptional processes to reduce FXN gene expression. Friedreich's ataxia, the genetic basis for the disease, and related symptoms are described in the art (see, *e.g.*, Montermini, L. et al. "The Friedreich's ataxia GAA triplet repeat: premutation and normal alleles." *Hum. Molec. Genet.*, 1997, 6: 1261-1266.; Filla, A. et al. "The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich's ataxia." *Am. J. Hum. Genet.* 1996, 59: 554-560.; Pandolfo, M. Friedreich's ataxia: the clinical picture. *J. Neurol.* 2009, 256, 3-8.) Friedreich's ataxia is associated with Online Mendelian Inheritance in Man (OMIM) Entry # 229300.

**[00047]      FXN:** As used herein, the term "FXN" refers to a gene that encodes frataxin, a protein implicated in iron homeostasis. In some embodiments, FXN may be a human (Gene ID: 2395), non-human primate (*e.g.*, Gene ID: 737660), or rodent gene (*e.g.*, Gene ID: 14297, Gene ID: 499335). In humans, a GAA repeat expansion in the first intron of FXN is associated with Friedreich's ataxia. In addition, multiple human transcript variants (*e.g.*, as annotated under GenBank RefSeq Accession Numbers: NM\_000144.4 and NM\_181425.2) have been characterized that encode different protein isoforms.

**[00048]      FXN allele:** As used herein, the term "FXN allele" refers to any one of alternative forms (*e.g.*, wild-type or mutant forms) of a FXN gene. In some embodiments, a FXN allele may encode for wild-type frataxin that retains its normal and typical functions. In some embodiments, a FXN allele may comprise one or more disease-associated-repeat

- 13 -

expansions. In some embodiments, normal subjects have two FXN alleles comprising in the range of 8 to 33 GAA trinucleotide repeat units. In some embodiments, the number of GAA repeat units in an FXN allele of subjects having Friedreich's ataxia is in the range of ~60 to ~1300 with higher numbers of repeats leading to an increased severity of disease. In some embodiments, mildly affected Friedreich's ataxia subjects have at least one FXN allele having in the range of 60 to 150 repeat units. In some embodiments, subjects with classic Friedreich's ataxia have at least one FXN allele having in the range of 90 to 1,000 or more repeat units.

**[00049] 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.

**[00050] 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 VL sequence has been altered to be more "human-like", *i.e.*, more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences. In one embodiment, humanized anti-transferrin receptor antibodies and antigen binding portions are provided. Such antibodies may be generated by obtaining murine anti-transferrin receptor monoclonal antibodies using traditional hybridoma technology followed by humanization using *in vitro* genetic engineering, such as those disclosed in Kasaian et al PCT publication No. WO 2005/123126 A2.

**[00051] Internalizing cell surface receptor:** As used herein, the term, "internalizing cell surface receptor" refers to a cell surface receptor that is internalized by cells, *e.g.*, upon external stimulation, *e.g.*, ligand binding to the receptor. In some embodiments, an internalizing cell surface receptor is internalized by endocytosis. In some embodiments, an internalizing cell surface receptor is internalized by clathrin-mediated endocytosis. However, in some embodiments, an internalizing cell surface receptor is internalized by a clathrin-independent

- 14 -

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

**[00052] 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 chemicals.

**[00053] Kabat numbering:** The terms "Kabat numbering", "Kabat definitions and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat et al. (1971) *Ann. NY Acad. Sci.* 190:382-391 and, Kabat, E. A., *et al.* (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

**[00054] Molecular payload:** As used herein, the term "molecular payload" refers to a molecule or species that functions to modulate a biological outcome. In some embodiments, a molecular payload is linked to, or otherwise associated with a muscle-targeting agent. In some embodiments, the molecular payload is a small molecule, a protein, a peptide, a nucleic acid, or an oligonucleotide. In some embodiments, the molecular payload functions to modulate the transcription of a DNA sequence, to modulate the expression of a protein, or to modulate the

- 15 -

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.

**[00055] Muscle-targeting agent:** As used herein, the term, “muscle-targeting agent,” refers to a molecule that specifically binds to an antigen expressed on muscle cells. The antigen in or on muscle cells may be a membrane protein, for example an integral membrane protein or a peripheral membrane protein. Typically, a muscle-targeting agent specifically binds to an antigen on muscle cells that facilitates internalization of the muscle-targeting agent (and any associated molecular payload) into the muscle cells. In some embodiments, a muscle-targeting agent specifically binds to an internalizing, cell surface receptor on muscles and is capable of being internalized into muscle cells through receptor mediated internalization. In some embodiments, the muscle-targeting agent is a small molecule, a protein, a peptide, a nucleic acid (*e.g.*, an aptamer), or an antibody. In some embodiments, the muscle-targeting agent is linked to a molecular payload.

**[00056] Muscle-targeting antibody:** As used herein, the term, “muscle-targeting antibody,” refers to a muscle-targeting agent that is an antibody that specifically binds to an antigen found in or on muscle cells. In some embodiments, a muscle-targeting antibody specifically binds to an antigen on muscle cells that facilitates internalization of the muscle-targeting antibody (and any associated molecular payment) into the muscle cells. In some embodiments, the muscle-targeting antibody specifically binds to an internalizing, cell surface receptor present on muscle cells. In some embodiments, the muscle-targeting antibody is an antibody that specifically binds to a transferrin receptor.

**[00057] 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.

- 16 -

**[00058] Recombinant antibody:** The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described in more details in this disclosure), antibodies isolated from a recombinant, combinatorial human antibody library (Hoogenboom H. R., (1997) TIB Tech. 15:62-70; Azzazy H., and Highsmith W. E., (2002) Clin. Biochem. 35:425-445; Gavilondo J. V., and Larrick J. W. (2002) BioTechniques 29:128-145; Hoogenboom H., and Chames P. (2000) Immunology Today 21:371-378), antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A., and Green L. L. (2002) Current Opinion in Biotechnology 13:593-597; Little M. et al (2000) Immunology Today 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. One embodiment of the disclosure provides fully human antibodies capable of binding human transferrin receptor which can be generated using techniques well known in the art, such as, but not limited to, using human Ig phage libraries such as those disclosed in Jermutus et al., PCT publication No. WO 2005/007699 A2.

**[00059] 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

- 17 -

complementarity contains 1, 2, 3, or 4 mismatches compared with a cognate nucleotide sequence of a target nucleic acid.

**[00060] Specifically binds:** As used herein, the term “specifically binds” refers to the ability of a molecule to bind to a binding partner with a degree of affinity or avidity that enables the molecule to be used to distinguish the binding partner from an appropriate control in a binding assay or other binding context. With respect to an antibody, the term, “specifically binds”, refers to the ability of the antibody to bind to a specific antigen with a degree of affinity or avidity, compared with an appropriate reference antigen or antigens, that enables the antibody to be used to distinguish the specific antigen from others, e.g., to an extent that permits preferential targeting to certain cells, e.g., muscle cells, through binding to the antigen, as described herein. In some embodiments, an antibody specifically binds to a target if the antibody has a  $K_D$  for binding the target of at least about  $10^{-4}$  M,  $10^{-5}$  M,  $10^{-6}$  M,  $10^{-7}$  M,  $10^{-8}$  M,  $10^{-9}$  M,  $10^{-10}$  M,  $10^{-11}$  M,  $10^{-12}$  M,  $10^{-13}$  M, or less. In some embodiments, an antibody specifically binds to the transferrin receptor, e.g., an epitope of the apical domain of transferrin receptor.

**[00061] 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 Friedreich’s ataxia. In some embodiments, the subject has an expanded GAA repeat in the FXN gene.

**[00062] Transferrin receptor:** As used herein, the term, “transferrin receptor” (also known as TFRC, CD71, p90, 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).

## II. Complexes

- 18 -

**[00063]** Provided herein are complexes that comprise a targeting agent, e.g. an antibody, covalently linked to a molecular payload. In some embodiments, a complex comprises a muscle-targeting antibody covalently linked to an oligonucleotide. A complex may comprise an antibody that specifically binds a single antigenic site or that binds to at least two antigenic sites that may exist on the same or different antigens.

**[00064]** A complex may be used to modulate the activity or function of at least one gene, protein, and/or nucleic acid. In some embodiments, the molecular payload present with a complex is responsible for the modulation of a gene, protein, and/or 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 nucleic acid in a cell. In some embodiments, a molecular payload is an oligonucleotide that targets FXN in muscle cells.

**[00065]** In some embodiments, a complex comprises a muscle-targeting agent, e.g. an anti-transferrin receptor antibody, covalently linked to a molecular payload, e.g. an antisense oligonucleotide that targets FXN.

#### **A. Muscle-Targeting Agents**

**[00066]** Some aspects of the disclosure provide muscle-targeting agents, *e.g.*, for delivering a molecular payload to a muscle cell. In some embodiments, such muscle-targeting agents are capable of binding to a muscle cell, *e.g.*, via specifically binding to an antigen on the muscle cell, and delivering an associated molecular payload to the muscle cell. In some embodiments, the molecular payload is bound (*e.g.*, covalently bound) to the muscle targeting agent and is internalized into the muscle cell upon binding of the muscle targeting agent to an antigen on the muscle cell, *e.g.*, via endocytosis. It should be appreciated that various types of muscle-targeting agents may be used in accordance with the disclosure. For example, the muscle-targeting agent may comprise, or consist of, a nucleic acid (*e.g.*, DNA or RNA), a peptide (*e.g.*, an antibody), a lipid (*e.g.*, a microvesicle), or a sugar moiety (*e.g.*, a polysaccharide). Exemplary muscle-targeting agents are described in further detail herein, however, it should be appreciated that the exemplary muscle-targeting agents provided herein are not meant to be limiting.

**[00067]** Some aspects of the disclosure provide muscle-targeting agents that specifically bind to an antigen on muscle, such as skeletal muscle, smooth muscle, or cardiac muscle. In

- 19 -

some embodiments, any of the muscle-targeting agents provided herein bind to (*e.g.*, specifically bind to) an antigen on a skeletal muscle cell, a smooth muscle cell, and/or a cardiac muscle cell.

**[00068]** By interacting with muscle-specific cell surface recognition elements (*e.g.*, cell membrane proteins), both tissue localization and selective uptake into muscle cells can be achieved. In some embodiments, molecules that are substrates for muscle uptake transporters are useful for delivering a molecular payload into muscle tissue. Binding to muscle surface recognition elements followed by endocytosis can allow even large molecules such as antibodies to enter muscle cells. As another example molecular payloads conjugated to transferrin or anti-transferrin receptor antibodies can be taken up by muscle cells via binding to transferrin receptor, which may then be endocytosed, *e.g.*, via clathrin-mediated endocytosis.

**[00069]** The use of muscle-targeting agents may be useful for concentrating a molecular payload (*e.g.*, oligonucleotide) in muscle while reducing toxicity associated with effects in other tissues. In some embodiments, the muscle-targeting agent concentrates a bound molecular payload in muscle cells as compared to another cell type within a subject. In some embodiments, the muscle-targeting agent concentrates a bound molecular payload in muscle cells (*e.g.*, skeletal, smooth, or cardiac muscle cells) in an amount that is at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times greater than an amount in non-muscle cells (*e.g.*, liver, neuronal, blood, or fat cells). In some embodiments, a toxicity of the molecular payload in a subject is reduced by at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90%, or 95% when it is delivered to the subject when bound to the muscle-targeting agent.

**[00070]** In some embodiments, to achieve muscle selectivity, a muscle recognition element (*e.g.*, a muscle cell antigen) may be required. As one example, a muscle-targeting agent may be a small molecule that is a substrate for a muscle-specific uptake transporter. As another example, a muscle-targeting agent may be an antibody that enters a muscle cell via transporter-mediated endocytosis. As another example, a muscle targeting agent may be a ligand that binds to cell surface receptor on a muscle cell. It should be appreciated that while transporter-based approaches provide a direct path for cellular entry, receptor-based targeting may involve stimulated endocytosis to reach the desired site of action.

#### **i. Muscle-Targeting Antibodies**

**[00071]** In some embodiments, the muscle-targeting agent is an antibody. Generally, the high specificity of antibodies for their target antigen provides the potential for selectively

- 20 -

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

**a. Anti-Transferrin Receptor Antibodies**

**[00072]** Some aspects of the disclosure are based on the recognition that agents binding to transferrin receptor, *e.g.*, anti-transferrin-receptor antibodies, are capable of targeting muscle cell. Transferrin receptors are internalizing cell surface receptors that transport transferrin across the cellular membrane and participate in the regulation and homeostasis of intracellular iron levels. Some aspects of the disclosure provide transferrin receptor binding proteins, which are capable of binding to transferrin receptor. Accordingly, aspects of the disclosure provide binding proteins (*e.g.*, antibodies) that bind to transferrin receptor. In some embodiments, binding proteins that bind to transferrin receptor are internalized, along with any bound molecular payload, into a muscle cell. As used herein, an antibody that binds to a transferrin receptor may be referred to as an anti-transferrin receptor antibody. Antibodies that bind, *e.g.* specifically bind, to a transferrin receptor may be internalized into the cell, *e.g.* through receptor-mediated endocytosis, upon binding to a transferrin receptor.

**[00073]** It should be appreciated that anti-transferrin receptor antibodies may be produced, synthesized, and/or derivatized using several known methodologies, *e.g.* library design using phage display. Exemplary methodologies have been characterized in the art and are incorporated by reference (Díez, P. et al. “High-throughput phage-display screening in array format”, *Enzyme and microbial technology*, 2015, 79, 34-41.; Christoph M. H. and Stanley, J.R. “Antibody Phage Display: Technique and Applications” *J Invest Dermatol.* 2014, 134:2.; Engleman, Edgar (Ed.) “Human Hybridomas and Monoclonal Antibodies.” 1985, Springer.). In other embodiments, an anti-transferrin antibody has been previously characterized or disclosed.

- 21 -

Antibodies that specifically bind to transferrin receptor are known in the art (see, e.g. US Patent. No. 4,364,934, filed 12/4/1979, “Monoclonal antibody to a human early thymocyte antigen and methods for preparing same”; US Patent No. 8,409,573, filed 6/14/2006, “Anti-CD71 monoclonal antibodies and uses thereof for treating malignant tumor cells”; US Patent No. 9,708,406, filed 5/20/2014, “Anti-transferrin receptor antibodies and methods of use”; US 9,611,323, filed 12/19/2014, “Low affinity blood brain barrier receptor antibodies and uses therefor”; WO 2015/098989, filed 12/24/2014, “Novel anti-Transferrin receptor antibody that passes through blood-brain barrier”; Schneider C. et al. “Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9.” J Biol Chem. 1982, 257:14, 8516-8522.; Lee et al. “Targeting Rat Anti-Mouse Transferrin Receptor Monoclonal Antibodies through Blood-Brain Barrier in Mouse” 2000, J Pharmacol. Exp. Ther., 292: 1048-1052.).

**[00074]** Any appropriate anti-transferrin receptor antibodies may be used in the complexes disclosed herein. Examples of anti-transferrin receptor antibodies, including associated references and binding epitopes, are listed in Table 1. In some embodiments, the anti-transferrin receptor antibody comprises the complementarity determining regions (CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3) of any of the anti-transferrin receptor antibodies provided herein, e.g., anti-transferrin receptor antibodies listed in Table 1.

**[00075]** Table 1 – List of anti-transferrin receptor antibody clones, including associated references and binding epitope information.

Antibody Clone Name	Reference(s)	Epitope / Notes
OKT9	US Patent. No. 4,364,934, filed 12/4/1979, entitled “MONOCLONAL ANTIBODY TO A HUMAN EARLY THYMOCYTE ANTIGEN AND METHODS FOR PREPARING SAME” Schneider C. et al. “Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9.” J Biol Chem. 1982, 257:14, 8516-8522.	Apical domain of TfR (residues 305-366 of human TfR sequence XM_052730.3, available in GenBank)
(From JCR) Clone M11 Clone M23 Clone M27	<ul style="list-style-type: none"> <li>WO 2015/098989, filed 12/24/2014, “Novel anti-Transferrin receptor antibody that passes through blood-brain barrier”</li> </ul>	Apical domain (residues 230-244 and 326-347 of TfR) and protease-like domain (residues 461-473)

- 22 -

Clone B84	<ul style="list-style-type: none"> <li>US Patent No. 9,994,641, filed 12/24/2014, "Novel anti-Transferrin receptor antibody that passes through blood-brain barrier"</li> </ul>	
(From Genentech)  7A4, 8A2, 15D2, 10D11, 7B10, 15G11, 16G5, 13C3, 16G4, 16F6, 7G7, 4C2, 1B12, and 13D4	<ul style="list-style-type: none"> <li>WO 2016/081643, filed 5/26/2016, entitled "ANTI-TRANSFERRIN RECEPTOR ANTIBODIES AND METHODS OF USE"</li> <li>US Patent No. 9,708,406, filed 5/20/2014, "Anti-transferrin receptor antibodies and methods of use"</li> </ul>	Apical domain and non-apical regions
(From Armagen)  8D3	<ul style="list-style-type: none"> <li>Lee et al. "Targeting Rat Anti-Mouse Transferrin Receptor Monoclonal Antibodies through Blood-Brain Barrier in Mouse" 2000, J Pharmacol. Exp. Ther., 292: 1048-1052.</li> <li>US Patent App. 2010/077498, filed 9/11/2008, entitled "COMPOSITIONS AND METHODS FOR BLOOD-BRAIN BARRIER DELIVERY IN THE MOUSE"</li> </ul>	
OX26	<ul style="list-style-type: none"> <li>Haobam, B. et al. 2014. Rab17-mediated recycling endosomes contribute to autophagosome formation in response to Group A Streptococcus invasion. Cellular microbiology. 16: 1806-21.</li> </ul>	
DF1513	<ul style="list-style-type: none"> <li>Ortiz-Zapater E et al. Trafficking of the human transferrin receptor in plant cells: effects of tyrphostin A23 and brefeldin A. Plant J 48:757-70 (2006).</li> </ul>	
1A1B2, 66IG10, MEM-189, JF0956, 29806, 1A1B2, TFRC/1818, 1E6, 66Ig10, TFRC/1059, Q1/71, 23D10, 13E4, TFRC/1149, ER-MP21, YTA74.4,	<ul style="list-style-type: none"> <li>Commercially available anti-transferrin receptor antibodies.</li> </ul>	Novus Biologicals 8100 Southpark Way, A-8 Littleton CO 80120

- 23 -

BU54, 2B6, RI7 217		
(From INSERM)  BA120g	<ul style="list-style-type: none"> <li>US Patent App. 2011/0311544A1, filed 6/15/2005, entitled "ANTI-CD71 MONOCLONAL ANTIBODIES AND USES THEREOF FOR TREATING MALIGNANT TUMOR CELLS"</li> </ul>	Does not compete with OKT9
LUCA31	<ul style="list-style-type: none"> <li>US Patent No. 7,572,895, filed 6/7/2004, entitled "TRANSFERRIN RECEPTOR ANTIBODIES"</li> </ul>	"LUCA31 epitope"
(Salk Institute)  B3/25 T58/30	<ul style="list-style-type: none"> <li>Trowbridge, I.S. et al. "Anti-transferrin receptor monoclonal antibody and toxin-antibody conjugates affect growth of human tumour cells." Nature, 1981, volume 294, pages 171-173</li> </ul>	
R17 217.1.3, 5E9C11, OKT9 (BE0023 clone)	<ul style="list-style-type: none"> <li>Commercially available anti-transferrin receptor antibodies.</li> </ul>	BioXcell 10 Technology Dr., Suite 2B West Lebanon, NH 03784-1671 USA
BK19.9, B3/25, T56/14 and T58/1	<ul style="list-style-type: none"> <li>Gatter, K.C. et al. "Transferrin receptors in human tissues: their distribution and possible clinical relevance." J Clin Pathol. 1983 May;36(5):539-45.</li> </ul>	

**[00076]** In some embodiments, the muscle-targeting agent is an anti-transferrin receptor antibody. In some embodiment, an anti-transferrin receptor antibody specifically binds to a transferrin protein having an amino acid sequence as disclosed herein. In some embodiments, an anti-transferrin receptor antibody may specifically bind to any extracellular epitope of a transferrin receptor or an epitope that becomes exposed to an antibody, including the apical domain, the transferrin binding domain, and the protease-like domain. In some embodiments, an anti-transferrin receptor antibody binds to an amino acid segment of a human or non-human primate transferrin receptor, as provided in SEQ ID Nos. 1-3 in the range of amino acids C89 to F760. In some embodiments, an anti-transferrin receptor antibody specifically binds with binding affinity 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. Anti-transferrin receptor antibodies used herein may be capable of competing for binding with other anti-transferrin receptor antibodies, e.g. OKT9, 8D3, that bind to transferrin receptor with  $10^{-3}$  M,  $10^{-4}$  M,  $10^{-5}$  M,  $10^{-6}$  M,  $10^{-7}$  M, or less.

- 24 -

**[00077]** 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:

MMDQARS AFSNLFGGEPLSYTRFSLARQVDGDN SHVEMKLA VDEEENADNNT  
KANVT KPKRCSGSICYGTIAVIVFFLIGFMIGYLG YCKGVEPKTECERLAGTESPVREEPG  
EDFPAARRLYWDDLKRKLSEKLDSTDFGTIKLLNENS YVPREAGSQKDENLALYVEN  
QFREFKLSKVWRDQHFVKIQVKDSAQNSVIIVDKNGRLVYLVENPGGYVAYS KAATVT  
GKLVHANFGTKKDFEDLYTPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKF  
PIVNAELSFFGHAHLGTGDPYTPGFPSFNHTQFPPSRSSGLPNIPVQTISR AAAEKLFGNM  
EGDCPSDWKTDSTCRMVTSESKNVKLTVSNVLKEIKILNIFGVIKGFVEPDHYVVVGAQ  
RDAWGPGA AAKSGVGTALLLKL AQMFSDMVLKDGFQPSRSIIFASWSAGDFG SVGATE  
WLEGYLSSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQNVKHPVTGQFLYQ  
DSNWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELIERIPELN  
KVARAAAEVAGQFVIKLT HDVELNLDYERYNSQLLSFVRDLNQYRADIKEMGLSLQW  
LYSARGDFFRATSRLTTDFGNAEKTDRFVMKKLNDRVMRVEYHFLSPYVSPKESPRH  
VFWGSGSHTLPALLENLKL RKQNGAFNETLFRNQLALATWTIQGAANALSGDVWDI  
DNEF (SEQ ID NO: 1).

**[00078]** 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:

MMDQARS AFSNLFGGEPLSYTRFSLARQVDGDN SHVEMKLG VDEEENTDNNTKPNGT  
KPKRCGGNICYGTIAVIIFFLIGFMIGYLG YCKGVEPKTECERLAGTESPAREEPEEDFPA  
APRLYWDDLKRKLSEKLDTTDFSTIKLLNENLYVPREAGSQKDENLALYIENQFREFK  
LSKVWRDQHFVKIQVKDSAQNSVIIVDKNGGLVYLVENPGGYVAYS KAATVTGKLVH  
ANFGTKKDFEDLDSPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVKAD  
LSFFGHAHLGTGDPYTPGFPSFNHTQFPPSQSSGLPNIPVQTISR AAAEKLFGNMEGDCPS  
DWKTDSTCKMVTSENKSVKLTVSNVLKETKILNIFGVIKGFVEPDHYVVVGAQRDAW  
GPGA AAKSSVGTALLLKL AQMFSDMVLKDGFQPSRSIIFASWSAGDFG SVGATEWLEGY  
LSSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQDVKHPVTGRSLYQDSNWA  
SKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELVERIPELNKVAR  
AAAEVAGQFVIKLT HDTELNLDYERYNSQLLLFLRDLNQYRADVKEMGLSLQWLYSA

- 25 -

RGDFFRATSRLTTDFRNAEKRDKFVMKKLNDRVMRVEYYFLSPYVSPKESPFRHVFHWG  
SGSHTLSALLESCLKLRRQNNSAFNETLFRNQLALATWTIQGAANALSGDVWDIDNEF  
(SEQ ID NO: 2)

**[00079]** 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:

MMDQARSASFNLFGGEPLSYTRFSLARQVDGDNSHVEMKLGVDDEENTDNNTKANGT  
KPKRCGGNICYGTIAVHIFFLIGFMIGYLYGYCKGVEPKTECERLAGTESPAREEPEEDFPA  
APRLYWDDLKRKLSEKLDTTDFTSTIKLLNENLYVPREAGSQKDENLALYIENQFREFK  
LSKVWRDQHFVKIQVKDSAQNSVIIVDKNGGLVYLVENPGGYVAYSKAATVTGKLVH  
ANFGTKKDFEDLDSPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVKAD  
LSFFGHAHLGTGDPYTPGFPSFNHTQFPPSQSSGLPNIPVQTISRAAAELFGNMEGDCPS  
DWKTDSTCKMVTSENKSVKLTVSNVLKETKILNIFGVIKGFVEPDHYVVVGAQRDAW  
GPGAACKSSVGTALLKLAQMFSDMVLKDGFPQRSIIFASWSAGDFGSGATEWLEGY  
LSSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQDVKHPVTGRSLYQDSNWA  
SKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELVERIPELNKVAR  
AAAEVAGQFVIKLTHTDELNDYERYNSQLLLFLRDLNQYRADVKEMGLSLQWLYSA  
RGDFFRATSRLTTDFRNAEKRDKFVMKKLNDRVMRVEYYFLSPYVSPKESPFRHVFHWG  
SGSHTLSALLESCLKLRRQNNSAFNETLFRNQLALATWTIQGAANALSGDVWDIDNEF  
(SEQ ID NO: 3).

**[00080]** An example mouse transferrin receptor amino acid sequence, corresponding to NCBI sequence NP\_001344227.1 (transferrin receptor protein 1, *mus musculus*) is as follows:

MMDQARSASFNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAADDEENADNNMKASV  
RKPKRFNGRLCFAAIALVIFFLIGFMSGYLYGYCKRVEQKEECVKLAETEETDKSETMETE  
DVPTSSRLYWADLKTLLSEKLNSEFADTIKQLSQNTYTPREAGSQKDESLAYYIENQFH  
EFKFSKVWRDEHYVKIQVKSSIGQNMVTIVQSNGNLDPVESPEGYVAFSKPTEVSGKLV  
HANFGTKKDFEELSYSVNGSLVIVRAGEITFAEKVANAQSFNAIGVLIYMDKNKFPVVE  
ADLALFGHAHLGTGDPYTPGFPSFNHTQFPPSQSSGLPNIPVQTISRAAAELFGKMEGS  
CPARWNIDSSCKLELSQNQNVLKIVKNVLKERRILNIFGVIKGYEEPDRYVVVGAQRDA  
LGAGVAAKSSVGTGLLLKLAQVFSDMISKDGRPSRSIIFASWTAGDFGAVGATEWLEG  
YLSSLHLKAFTYINLDKVVLGTSNFKVSASPLLYTLMGKIMQDVKHPVDGKSLYRDSN  
WISKVEKLSFDNAAYPFLAYSGIPAVSFCFCEDADYPYLGTRLDTYEALTQKVPQLNQM

- 26 -

VRTAAEVAGQLIIKLTHDVELNLDYEMYSKLLSFMKDLNQFKTDIRDMGLSLQWLYS  
ARGDYFRATSRLTTDFHNAEKTNRFMREINDRIMKVEYHFLSPYVSPRESPFRHIFWG  
SGSHTLSALVENLKLQRKNITAFNETLFRNQLALATWTIQGVANALSGDIWNIDNEF  
(SEQ ID NO: 4)

In some embodiments, an anti-transferrin receptor antibody binds to an amino acid segment of the receptor as follows:

FVKIQVKDSAQNSVIVDKNGRLVYLVENPGGYVAYSKAATVTGKLVHANFGTKKDFE  
DLYTPVNGSIVIVRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVNAELFFGHHLG  
TGDPYTPGFPSFNHTQPPSRSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWKTDSTCR  
MVTSESKNVKLTVSNVLKE (SEQ ID NO: 5) and does not inhibit the binding interactions between transferrin receptors and transferrin and/or human hemochromatosis protein (also known as HFE).

**[00081]** Appropriate methodologies may be used to obtain and/or produce antibodies, antibody fragments, or antigen-binding agents, e.g., through the use of recombinant DNA protocols. In some embodiments, an antibody may also be produced through the generation of hybridomas (see, e.g., Kohler, G and Milstein, C. “Continuous cultures of fused cells secreting antibody of predefined specificity” *Nature*, 1975, 256: 495-497). The antigen-of-interest may be used as the immunogen in any form or entity, e.g., recombinant or a naturally occurring form or entity. Hybridomas are screened using standard methods, e.g. ELISA screening, to find at least one hybridoma that produces an antibody that targets a particular antigen. Antibodies may also be produced through screening of protein expression libraries that express antibodies, e.g., phage display libraries. Phage display library design may also be used, in some embodiments, (see, e.g. U.S. Patent No 5,223,409, filed 3/1/1991, “Directed evolution of novel binding proteins”; WO 1992/18619, filed 4/10/1992, “Heterodimeric receptor libraries using phagemids”; WO 1991/17271, filed 5/1/1991, “Recombinant library screening methods”; WO 1992/20791, filed 5/15/1992, “Methods for producing members of specific binding pairs”; WO 1992/15679, filed 2/28/1992, and “Improved epitope displaying phage”). In some embodiments, an antigen-of-interest may be used to immunize a non-human animal, e.g., a rodent or a goat. In some embodiments, an antibody is then obtained from the non-human animal, and may be optionally modified using a number of methodologies, e.g., using recombinant DNA techniques. Additional examples of antibody production and methodologies are known in the art (see, e.g. Harlow et al. “Antibodies: A Laboratory Manual”, Cold Spring Harbor Laboratory, 1988.).

- 27 -

**[00082]** In some embodiments, an antibody is modified, e.g., modified via glycosylation, phosphorylation, sumoylation, and/or 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 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*”.

**[00083]** Some aspects of the disclosure provide proteins that bind to transferrin receptor (e.g., an extracellular portion of the transferrin receptor). In some embodiments, transferrin receptor antibodies provided herein bind specifically to transferrin receptor (e.g., human transferrin receptor). 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. In some embodiments, transferrin receptor antibodies provided herein bind specifically to transferrin receptor from human, non-human primates, mouse, rat, *etc.* In some embodiments, transferrin receptor antibodies provided herein bind to human transferrin receptor. In some embodiments, transferrin receptor antibodies provided herein specifically bind to human transferrin receptor. In some embodiments, transferrin receptor antibodies provided herein bind to an apical domain of human transferrin receptor. In some embodiments, transferrin

- 28 -

receptor antibodies provided herein specifically bind to an apical domain of human transferrin receptor.

**[00084]** In some embodiments, transferrin receptor antibodies of the present disclosure include one or more of the CDR-H (*e.g.*, CDR-H1, CDR-H2, and CDR-H3) amino acid sequences from any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, transferrin receptor antibodies include the CDR-H1, CDR-H2, and CDR-H3 as provided for any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, anti-transferrin receptor antibodies include the CDR-L1, CDR-L2, and CDR-L3 as provided for any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, anti-transferrin antibodies include the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3 as provided for any one of the anti-transferrin receptor antibodies selected from Table 1. The disclosure also includes any nucleic acid sequence that encodes a molecule comprising a CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, or CDR-L3 as provided for any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, antibody heavy and light chain CDR3 domains may play a particularly important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, anti-transferrin receptor antibodies of the disclosure may include at least the heavy and/or light chain CDR3s of any one of the anti-transferrin receptor antibodies selected from Table 1.

**[00085]** In some examples, any of the anti-transferrin receptor antibodies of the disclosure have one or more CDR (*e.g.*, CDR-H or CDR-L) sequences substantially similar to any of the CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and/or CDR-L3 sequences from one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, the position of one or more CDRs along the VH (*e.g.*, CDR-H1, CDR-H2, or CDR-H3) and/or VL (*e.g.*, CDR-L1, CDR-L2, or CDR-L3) region of an antibody described herein can vary by one, two, three, four, five, or six amino acid positions so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% of the binding of the original antibody from which it is derived). For example, in some embodiments, the position defining a CDR of any antibody described herein can vary by shifting the N-terminal and/or C-terminal boundary of the CDR by one, two, three, four, five, or six amino acids, relative to the CDR position of any one of the antibodies described herein, so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is

- 29 -

maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% of the binding of the original antibody from which it is derived). In another embodiment, the length of one or more CDRs along the VH (*e.g.*, CDR-H1, CDR-H2, or CDR-H3) and/or VL (*e.g.*, CDR-L1, CDR-L2, or CDR-L3) region of an antibody described herein can vary (*e.g.*, be shorter or longer) by one, two, three, four, five, or more amino acids, so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% of the binding of the original antibody from which it is derived).

**[00086]** Accordingly, in some embodiments, a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or CDR-H3 described herein may be one, two, three, four, five or more amino acids shorter than one or more of the CDRs described herein (*e.g.*, CDRs from any of the anti-transferrin receptor antibodies selected from Table 1) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or CDR-H3 described herein may be one, two, three, four, five or more amino acids longer than one or more of the CDRs described herein (*e.g.*, CDRs from any of the anti-transferrin receptor antibodies selected from Table 1) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, the amino portion of a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or CDR-H3 described herein can be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, CDRs from any of the anti-transferrin receptor antibodies selected from Table 1) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, the carboxy portion of a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or CDR-H3 described herein can be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, CDRs from any

- 30 -

of the anti-transferrin receptor antibodies selected from Table 1) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, the amino portion of a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or CDR-H3 described herein can be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, CDRs from any of the anti-transferrin receptor antibodies selected from Table 1) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, the carboxy portion of a CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and/or CDR-H3 described herein can be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described herein (*e.g.*, CDRs from any of the anti-transferrin receptor antibodies selected from Table 1) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). Any method can be used to ascertain whether immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained, for example, using binding assays and conditions described in the art.

**[00087]** In some examples, any of the anti-transferrin receptor antibodies of the disclosure have one or more CDR (*e.g.*, CDR-H or CDR-L) sequences substantially similar to any one of the anti-transferrin receptor antibodies selected from Table 1. For example, the antibodies may include one or more CDR sequence(s) from any of the anti-transferrin receptor antibodies selected from Table 1 containing up to 5, 4, 3, 2, or 1 amino acid residue variations as compared to the corresponding CDR region in any one of the CDRs provided herein (*e.g.*, CDRs from any of the anti-transferrin receptor antibodies selected from Table 1) so long as immunospecific binding to transferrin receptor (*e.g.*, human transferrin receptor) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95% relative to the binding of the original antibody from which it is derived). In some embodiments, any of the amino acid variations in any of the CDRs provided herein may be

- 31 -

conservative variations. Conservative variations can be introduced into the CDRs at positions where the residues are not likely to be involved in interacting with a transferrin receptor protein (*e.g.*, a human transferrin receptor protein), for example, as determined based on a crystal structure. Some aspects of the disclosure provide transferrin receptor antibodies that comprise one or more of the heavy chain variable (VH) and/or light chain variable (VL) domains provided herein. In some embodiments, any of the VH domains provided herein include one or more of the CDR-H sequences (*e.g.*, CDR-H1, CDR-H2, and CDR-H3) provided herein, for example, any of the CDR-H sequences provided in any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, any of the VL domains provided herein include one or more of the CDR-L sequences (*e.g.*, CDR-L1, CDR-L2, and CDR-L3) provided herein, for example, any of the CDR-L sequences provided in any one of the anti-transferrin receptor antibodies selected from Table 1.

**[00088]** In some embodiments, anti-transferrin receptor antibodies of the disclosure include any antibody that includes a heavy chain variable domain and/or a light chain variable domain of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, anti-transferrin receptor antibodies of the disclosure include any antibody that includes the heavy chain variable and light chain variable pairs of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1.

**[00089]** Aspects of the disclosure provide anti-transferrin receptor antibodies having a heavy chain variable (VH) and/or a light chain variable (VL) domain amino acid sequence homologous to any of those described herein. In some embodiments, the anti-transferrin receptor antibody comprises a heavy chain variable sequence or a light chain variable sequence that is at least 75% (*e.g.*, 80%, 85%, 90%, 95%, 98%, or 99%) identical to the heavy chain variable sequence and/ or any light chain variable sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, the homologous heavy chain variable and/or a light chain variable amino acid sequences do not vary within any of the CDR sequences provided herein. For example, in some embodiments, the degree of sequence variation (*e.g.*, 75%, 80%, 85%, 90%, 95%, 98%, or 99%) may occur within a heavy chain variable and/or a light chain variable sequence excluding any of the CDR sequences provided herein. In some embodiments, any of the anti-transferrin receptor antibodies provided herein comprise a heavy chain variable sequence and a light chain

- 32 -

variable sequence that comprises a framework sequence that is at least 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the framework sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1.

**[00090]** In some embodiments, an anti-transferrin receptor antibody, which specifically binds to transferrin receptor (*e.g.*, human transferrin receptor), comprises a light chain variable VL domain comprising any of the CDR-L domains (CDR-L1, CDR-L2, and CDR-L3), or CDR-L domain variants provided herein, of any of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, an anti-transferrin receptor antibody, which specifically binds to transferrin receptor (*e.g.*, human transferrin receptor), comprises a light chain variable VL domain comprising the CDR-L1, the CDR-L2, and the CDR-L3 of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, the anti-transferrin receptor antibody comprises a light chain variable (VL) region sequence comprising one, two, three or four of the framework regions of the light chain variable region sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, the anti-transferrin receptor antibody comprises one, two, three or four of the framework regions of a light chain variable region sequence which is at least 75%, 80%, 85%, 90%, 95%, or 100% identical to one, two, three or four of the framework regions of the light chain variable region sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, the light chain variable framework region that is derived from said amino acid sequence consists of said amino acid sequence but for the presence of up to 10 amino acid substitutions, deletions, and/or insertions, preferably up to 10 amino acid substitutions. In some embodiments, the light chain variable framework region that is derived from said amino acid sequence consists of said amino acid sequence with 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid residues being substituted for an amino acid found in an analogous position in a corresponding non-human, primate, or human light chain variable framework region.

**[00091]** In some embodiments, an anti-transferrin receptor antibody that specifically binds to transferrin receptor comprises the CDR-L1, the CDR-L2, and the CDR-L3 of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, the antibody further comprises one, two, three or all four

- 33 -

VL framework regions derived from the VL of a human or primate antibody. The primate or human light chain framework region of the antibody selected for use with the light chain CDR sequences described herein, can have, for example, at least 70% (*e.g.*, at least 75%, 80%, 85%, 90%, 95%, 98%, or at least 99%) identity with a light chain framework region of a non-human parent antibody. The primate or human antibody selected can have the same or substantially the same number of amino acids in its light chain complementarity determining regions to that of the light chain complementarity determining regions of any of the antibodies provided herein, *e.g.*, any of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, the primate or human light chain framework region amino acid residues are from a natural primate or human antibody light chain framework region having at least 75% identity, at least 80% identity, at least 85% identity, at least 90% identity, at least 95% identity, at least 98% identity, at least 99% (or more) identity with the light chain framework regions of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1. In some embodiments, an anti-transferrin receptor antibody further comprises one, two, three or all four VL framework regions derived from a human light chain variable kappa subfamily. In some embodiments, an anti-transferrin receptor antibody further comprises one, two, three or all four VL framework regions derived from a human light chain variable lambda subfamily.

**[00092]** In some embodiments, any of the anti-transferrin receptor antibodies provided herein comprise a light chain variable domain that further comprises a light chain constant region. In some embodiments, the light chain constant region is a kappa, or a lambda light chain constant region. In some embodiments, the kappa or lambda light chain constant region is from a mammal, *e.g.*, from a human, monkey, rat, or mouse. In some embodiments, the light chain constant region is a human kappa light chain constant region. In some embodiments, the light chain constant region is a human lambda light chain constant region. It should be appreciated that any of the light chain constant regions provided herein may be variants of any of the light chain constant regions provided herein. In some embodiments, the light chain constant region comprises an amino acid sequence that is at least 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to any of the light chain constant regions of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1.

- 34 -

**[00093]** In some embodiments, the anti-transferrin receptor antibody is any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1.

**[00094]** In some embodiments, an anti-transferrin receptor antibody comprises a VL domain comprising the amino acid sequence of any anti-transferrin receptor antibody, such as any one of the anti-transferrin receptor antibodies selected from Table 1, and wherein the constant regions comprise the amino acid sequences of the constant regions of an IgG, IgE, IgM, IgD, IgA or IgY immunoglobulin molecule, or a human IgG, IgE, IgM, IgD, IgA or IgY immunoglobulin molecule. In some embodiments, an anti-transferrin receptor antibody comprises any of the VL domains, or VL domain variants, and any of the VH domains, or VH domain variants, wherein the VL and VH domains, or variants thereof, are from the same antibody clone, and wherein the constant regions comprise the amino acid sequences of the constant regions of an IgG, IgE, IgM, IgD, IgA or IgY immunoglobulin molecule, any class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), or any subclass (e.g., IgG2a and IgG2b) of immunoglobulin molecule. Non-limiting examples of human constant regions are described in the art, e.g., see Kabat E A et al., (1991) supra.

**[00095]** In some embodiments, an antibody of the disclosure can bind to a target antigen (e.g., transferrin receptor) with relatively high affinity, e.g., with a  $K_D$  less than  $10^{-6}$  M,  $10^{-7}$  M,  $10^{-8}$  M,  $10^{-9}$  M,  $10^{-10}$  M,  $10^{-11}$  M or lower. For example, anti-transferrin receptor antibodies can bind to a transferrin receptor protein (e.g., human transferrin receptor) with an affinity between 5 pM and 500 nM, e.g., between 50 pM and 100 nM, e.g., between 500 pM and 50 nM. The disclosure also includes antibodies that compete with any of the antibodies described herein for binding to a transferrin receptor protein (e.g., human transferrin receptor) and that have an affinity of 50 nM or lower (e.g., 20 nM or lower, 10 nM or lower, 500 pM or lower, 50 pM or lower, or 5 pM or lower). The affinity and binding kinetics of the anti-transferrin receptor antibody can be tested using any suitable method including but not limited to biosensor technology (e.g., OCTET or BIACORE).

**[00096]** In some embodiments, an antibody of the disclosure can bind to a target antigen (e.g., transferrin receptor) with relatively high affinity, e.g., with a  $K_D$  less than  $10^{-6}$  M,  $10^{-7}$  M,  $10^{-8}$  M,  $10^{-9}$  M,  $10^{-10}$  M,  $10^{-11}$  M or lower. For example, anti-transferrin receptor antibodies can bind to a transferrin receptor protein (e.g., human transferrin receptor) with an affinity between 5 pM and 500 nM, e.g., between 50 pM and 100 nM, e.g., between 500 pM and 50 nM. The

- 35 -

disclosure also includes antibodies that compete with any of the antibodies described herein for binding to a transferrin receptor protein (*e.g.*, human transferrin receptor) and that have an affinity of 50 nM or lower (*e.g.*, 20 nM or lower, 10 nM or lower, 500 pM or lower, 50 pM or lower, or 5 pM or lower). The affinity and binding kinetics of the anti-transferrin receptor antibody can be tested using any suitable method including but not limited to biosensor technology (*e.g.*, OCTET or BIACORE).

**[00097]** In some embodiments, the muscle-targeting agent is a transferrin receptor antibody (*e.g.*, the antibody and variants thereof as described in International Application Publication WO 2016/081643, incorporated herein by reference).

**[00098]** The heavy chain and light chain CDRs of the antibody according to different definition systems are provided in Table 1.1. The different definition systems, *e.g.*, the Kabat definition, the Chothia definition, and/or the contact definition have been described. See, *e.g.*, (*e.g.*, Kabat, E.A., et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, Chothia et al., (1989) Nature 342:877; Chothia, C. et al. (1987) J. Mol. Biol. 196:901-917, Al-lazikani et al (1997) J. Molec. Biol. 273:927-948; and Almagro, J. Mol. Recognit. 17:132-143 (2004). See also hgmp.mrc.ac.uk and bioinf.org.uk/abs).

Table 1.1 Heavy chain and light chain CDRs of a mouse transferrin receptor antibody

CDRs	Kabat	Chothia	Contact
CDR-H1	SYWMH (SEQ ID NO: 17)	GYTFTSY (SEQ ID NO: 23)	TSYWMH (SEQ ID NO: 25)
CDR-H2	EINPTNGRTNYIEKFKS (SEQ ID NO: 18)	NPTNGR (SEQ ID NO: 24)	WIGEINPTNGRTN (SEQ ID NO: 26)
CDR-H3	GTRAYHY (SEQ ID NO: 19)	GTRAYHY (SEQ ID NO: 19)	ARGTRA (SEQ ID NO: 27)
CDR-L1	RASDNLYSNLA	RASDNLYSNLA	YSNLAWY

- 36 -

	(SEQ ID NO: 20)	(SEQ ID NO: 20)	(SEQ ID NO: 28)
CDR-L2	DATNLAD (SEQ ID NO: 21)	DATNLAD (SEQ ID NO: 21)	LLVYDATNLA (SEQ ID NO: 29)
CDR-L3	QHFWGTPLT (SEQ ID NO: 22)	QHFWGTPLT (SEQ ID NO: 22)	QHFWGTPL (SEQ ID NO: 30)

**[00099]** The heavy chain variable domain (VH) and light chain variable domain sequences are also provided:

**[000100]** VH

QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGEINPTNGR  
TNYIEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARGTRAYHYWGQGTSVTVS  
S (SEQ ID NO: 33)

**[000101]** VL

DIQMTQSPASLSVSVGETVTITCRASDNLYSNLAWYQQKQGKSPQLLVYDATNLADGV  
PSRFSGSGSGTQYSLKINSLQSEDFGTYYCQHFWGTPLTFGAGTKLELK (SEQ ID NO:  
34)

**[000102]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, and a CDR-H3 that are the same as the CDR-H1, CDR-H2, and CDR-H3 shown in Table 1.1. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a CDR-L1, a CDR-L2, and a CDR-L3 that are the same as the CDR-L1, CDR-L2, and CDR-L3 shown in Table 1.1.

**[000103]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, and a CDR-H3, which collectively contains no more than 5 amino acid variations (e.g., no more than 5, 4, 3, 2, or 1 amino acid variation) as compared with the CDR-H1, CDR-H2, and CDR-H3 as shown in Table 1.1. "Collectively" means that the total number of amino acid variations in all of the three heavy chain CDRs is within the defined range. Alternatively or in addition, the transferrin receptor antibody of the present disclosure may comprise a CDR-L1, a CDR-L2, and a CDR-L3, which collectively contains no more than

- 37 -

5 amino acid variations (e.g., no more than 5, 4, 3, 2 or 1 amino acid variation) as compared with the CDR-L1, CDR-L2, and CDR-L3 as shown in Table 1.1.

**[000104]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, and a CDR-H3, at least one of which contains no more than 3 amino acid variations (e.g., no more than 3, 2, or 1 amino acid variation) as compared with the counterpart heavy chain CDR as shown in Table 1.1. Alternatively or in addition, the transferrin receptor antibody of the present disclosure may comprise CDR-L1, a CDR-L2, and a CDR-L3, at least one of which contains no more than 3 amino acid variations (e.g., no more than 3, 2, or 1 amino acid variation) as compared with the counterpart light chain CDR as shown in Table 1.1.

**[000105]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-L3, which contains no more than 3 amino acid variations (e.g., no more than 3, 2, or 1 amino acid variation) as compared with the CDR-L3 as shown in Table 1.1. In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-L3 containing one amino acid variation as compared with the CDR-L3 as shown in Table 1.1. In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-L3 of QHFAGTPLT (SEQ ID NO: 31 according to the Kabat and Chothia definition system) or QHFAGTPL (SEQ ID NO: 32 according to the Contact definition system). In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1 and a CDR-L2 that are the same as the CDR-H1, CDR-H2, and CDR-H3 shown in Table 1.1, and comprises a CDR-L3 of QHFAGTPLT (SEQ ID NO: 31 according to the Kabat and Chothia definition system) or QHFAGTPL (SEQ ID NO: 32 according to the Contact definition system).

**[000106]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises heavy chain CDRs that collectively are at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the heavy chain CDRs as shown in Table 1.1. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises light chain CDRs that collectively are at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the light chain CDRs as shown in Table 1.1.

**[000107]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH comprising the amino acid sequence of SEQ ID NO: 33. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a VL comprising the amino acid sequence of SEQ ID NO: 34.

- 38 -

**[000108]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH containing no more than 20 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VH as set forth in SEQ ID NO: 33. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a VL containing no more than 15 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VL as set forth in SEQ ID NO: 34.

**[000109]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the VH as set forth in SEQ ID NO: 33. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a VL comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the VL as set forth in SEQ ID NO: 34.

**[000110]** In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized antibody (e.g., a humanized variant of an antibody). In some embodiments, the transferrin receptor antibody of the present disclosure comprises a CDR-H1, a CDR-H2, a CDR-H3, a CDR-L1, a CDR-L2, and a CDR-L3 that are the same as the CDR-H1, CDR-H2, and CDR-H3 shown in Table 1.1, and comprises a humanized heavy chain variable region and/or a humanized light chain variable region.

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

- 39 -

Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs derived from one or more CDRs from the original antibody. Humanized antibodies may also involve affinity maturation.

**[000112]** In some embodiments, humanization is achieved by grafting the CDRs (e.g., as shown in Table 1.1) into the IGKV1-NL1\*01 and IGHV1-3\*01 human variable domains. In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising one or more amino acid substitutions at positions 9, 13, 17, 18, 40, 45, and 70 as compared with the VL as set forth in SEQ ID NO: 34, and/or one or more amino acid substitutions at positions 1, 5, 7, 11, 12, 20, 38, 40, 44, 66, 75, 81, 83, 87, and 108 as compared with the VH as set forth in SEQ ID NO: 33. In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising amino acid substitutions at all of positions 9, 13, 17, 18, 40, 45, and 70 as compared with the VL as set forth in SEQ ID NO: 34, and/or amino acid substitutions at all of positions 1, 5, 7, 11, 12, 20, 38, 40, 44, 66, 75, 81, 83, 87, and 108 as compared with the VH as set forth in SEQ ID NO: 33.

**[000113]** In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized antibody and contains the residues at positions 43 and 48 of the VL as set forth in SEQ ID NO: 34. Alternatively or in addition, the transferrin receptor antibody of the present disclosure is a humanized antibody and contains the residues at positions 48, 67, 69, 71, and 73 of the VH as set forth in SEQ ID NO: 33.

**[000114]** The VH and VL amino acid sequences of an example humanized antibody that may be used in accordance with the present disclosure are provided:

**[000115]** Humanized VH

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQRLEWIGEINPTNGR  
TNYIEKFKSRATLTVDKSASTAYMELSSLRSEDTAVYYCARGTRAYHYWGQGTMVTV  
SS (SEQ ID NO: 35)

**[000116]** Humanized VL

DIQMTQSPSSLSASVGDRVTITCRASDNLYSNLAWYQQKPGKSPKLLVYDATNLADGV  
PSRFGSGSGTDYSLKINSLSQSEDFGTYCYCQHFHWGTPLTFGAGTKLELK (SEQ ID NO:  
36)

- 40 -

**[000117]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH comprising the amino acid sequence of SEQ ID NO: 35. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a VL comprising the amino acid sequence of SEQ ID NO: 36.

**[000118]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH containing no more than 20 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VH as set forth in SEQ ID NO: 35. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a VL containing no more than 15 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the VL as set forth in SEQ ID NO: 36.

**[000119]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a VH comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the VH as set forth in SEQ ID NO: 35. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a VL comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to the VL as set forth in SEQ ID NO: 36.

**[000120]** In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising amino acid substitutions at one or more of positions 43 and 48 as compared with the VL as set forth in SEQ ID NO: 34, and/or amino acid substitutions at one or more of positions 48, 67, 69, 71, and 73 as compared with the VH as set forth in SEQ ID NO: 33. In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising a S43A and/or a V48L mutation as compared with the VL as set forth in SEQ ID NO: 34, and/or one or more of A67V, L69I, V71R, and K73T mutations as compared with the VH as set forth in SEQ ID NO: 33.

**[000121]** In some embodiments, the transferrin receptor antibody of the present disclosure is a humanized variant comprising amino acid substitutions at one or more of positions 9, 13, 17, 18, 40, 43, 48, 45, and 70 as compared with the VL as set forth in SEQ ID NO: 34, and/or amino acid substitutions at one or more of positions 1, 5, 7, 11, 12, 20, 38, 40, 44, 48, 66, 67, 69, 71, 73, 75, 81, 83, 87, and 108 as compared with the VH as set forth in SEQ ID NO: 33.

**[000122]** In some embodiments, the transferrin receptor antibody of the present disclosure is a chimeric antibody, which can include a heavy constant region and a light constant region

- 41 -

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

**[000123]** In some embodiments, the transferrin receptor antibody described herein is a chimeric antibody, which can include a heavy constant region and a light constant region from a human antibody. Chimeric antibodies refer to antibodies having a variable region or part of variable region from a first species and a constant region from a second species. Typically, in these chimeric antibodies, the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals (e.g., a non-human mammal such as mouse, rabbit, and rat), while the constant portions are homologous to the sequences in antibodies derived from another mammal such as human. In some embodiments, amino acid modifications can be made in the variable region and/or the constant region.

**[000124]** In some embodiments, the heavy chain of any of the transferrin receptor antibodies as described herein may comprises a heavy chain constant region (CH) or a portion thereof (e.g., CH1, CH2, CH3, or a combination thereof). The heavy chain constant region can of any suitable origin, e.g., human, mouse, rat, or rabbit. In one specific example, the heavy chain constant region is from a human IgG (a gamma heavy chain), e.g., IgG1, IgG2, or IgG4. An exemplary human IgG1 constant region is given below:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 37)

**[000125]** In some embodiments, the light chain of any of the transferrin receptor antibodies described herein may further comprise a light chain constant region (CL), which can be any CL known in the art. In some examples, the CL is a kappa light chain. In other examples, the CL is a

- 42 -

lambda light chain. In some embodiments, the CL is a kappa light chain, the sequence of which is provided below:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP (SEQ ID NO: 38)

**[000126]** 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](http://www.imgt.org)) or at [www.vbase2.org/vbstat.php](http://www.vbase2.org/vbstat.php), both of which are incorporated by reference herein.

**[000127]** Exemplary heavy chain and light chain amino acid sequences of the transferrin receptor antibodies described are provided below:

**[000128]** Heavy Chain (VH + human IgG1 constant region)

QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGEINPTNGR  
TNYIEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARGTRAYHYWGQGTSVTVS  
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL  
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE  
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL  
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT  
VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 39)

**[000129]** Light Chain (VL + kappa light chain)

QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGEINPTNGR  
TNYIEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARGTRAYHYWGQGTSVTVS  
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP (SEQ ID  
NO: 40)

**[000130]** Heavy Chain (humanized VH + human IgG1 constant region)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQRLEWIGEINPTNGR  
TNYIEKFKSRATLTVDKSASTAYMELSSLRSEDTAVYYCARGTRAYHYWGQGTMTVTV  
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL  
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR  
EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT

- 43 -

LPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL  
TVDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 41)

**[000131]** Light Chain (humanized VL + kappa light chain)

DIQMTQSPSSLSASVGDRVTITCRASDNLYSNLAWYQQKPGKSPKLLVYDATNLADGV  
PSRFGSGSGTDYSLKINSLSQSEDFGTYYCQHFHWGTPFTFGAGTKLELKASTKGPSVFPL  
APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT  
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP (SEQ ID NO: 42)

**[000132]** In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 39. Alternatively or in addition, the transferrin receptor antibody described herein comprises a light chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 40. In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 39. Alternatively or in addition, the transferrin receptor antibody described herein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 40.

**[000133]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a heavy chain containing no more than 20 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the heavy chain as set forth in SEQ ID NO: 39. Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a light chain containing no more than 15 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the light chain as set forth in SEQ ID NO: 40.

**[000134]** In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 41. Alternatively or in addition, the transferrin receptor antibody described herein comprises a light chain comprising an amino acid sequence that is at least 80% (e.g., 80%, 85%, 90%, 95%, or 98%) identical to SEQ ID NO: 42. In some embodiments, the transferrin receptor antibody described herein comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 41. Alternatively or in addition, the

- 44 -

transferrin receptor antibody described herein comprises a light chain comprising the amino acid sequence of SEQ ID NO: 42.

**[000135]** In some embodiments, the transferrin receptor antibody of the present disclosure comprises a heavy chain containing no more than 20 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the heavy chain of humanized antibody as set forth in SEQ ID NO: 39.

Alternatively or in addition, the transferrin receptor antibody of the present disclosure comprises a light chain containing no more than 15 amino acid variations (e.g., no more than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 9, 8, 7, 6, 5, 4, 3, 2, or 1 amino acid variation) as compared with the light chain of humanized antibody as set forth in SEQ ID NO: 40.

**[000136]** In some embodiments, the transferrin receptor antibody is an antigen binding fragment (FAB) of an intact antibody (full-length antibody). Antigen binding fragment of an intact antibody (full-length antibody) can be prepared via routine methods. For example, F(ab')<sub>2</sub> 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')<sub>2</sub> fragments. Exemplary FABs amino acid sequences of the transferrin receptor antibodies described herein are provided below:

**[000137]** Heavy Chain FAB (VH + a portion of human IgG1 constant region)

QVQLQQPGAELVKPGASVKLSCKASGYTFTSYWMHWVKQRPGQGLEWIGEINPTNGR  
TNYIEKFKSKATLTVDKSSSTAYMQLSSLTSEDSAVYYCARGTRAYHYWGQGTSTVTVS  
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP (SEQ ID  
NO: 43)

**[000138]** Heavy Chain FAB (humanized VH + a portion of human IgG1 constant region)

EVQLVQSGAEVKKPGASVKVSCKASGYTFTSYWMHWVRQAPGQRLEWIGEINPTNGR  
TNYIEKFKSRATLTVDKSASTAYMELSSLRSEDTAVYYCARGTRAYHYWGQGTMTVTV  
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL  
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP (SEQ ID  
NO: 44)

**[000139]** The transferrin receptor antibodies described herein can be in any antibody form, including, but not limited to, intact (i.e., full-length) antibodies, antigen-binding fragments thereof (such as Fab, Fab', F(ab')<sub>2</sub>, Fv), single chain antibodies, bi-specific antibodies, or nanobodies. In some embodiments, the transferrin receptor antibody described herein is a scFv.

- 45 -

In some embodiments, the transferrin receptor antibody described herein is a scFv-Fab (e.g., scFv fused to a portion of a constant region). In some embodiments, the transferrin receptor antibody described herein is a scFv fused to a constant region (e.g., human IgG1 constant region as set forth in SEQ ID NO: 39).

### **b. Other Muscle-Targeting Antibodies**

**[000140]** In some embodiments, the muscle-targeting antibody is an antibody that specifically binds hemojuvelin, caveolin-3, Duchenne muscular dystrophy peptide, myosin Iib or CD63. In some embodiments, the muscle-targeting antibody is an antibody that specifically binds a myogenic precursor protein. Exemplary myogenic precursor proteins include, without limitation, ABCG2, M-Cadherin/Cadherin-15, Caveolin-1, CD34, FoxK1, Integrin alpha 7, Integrin alpha 7 beta 1, MYF-5, MyoD, Myogenin, NCAM-1/CD56, Pax3, Pax7, and Pax9. In some embodiments, the muscle-targeting antibody is an antibody that specifically binds a skeletal muscle protein. Exemplary skeletal muscle proteins include, without limitation, alpha-Sarcoglycan, beta-Sarcoglycan, Calpain Inhibitors, Creatine Kinase MM/CKMM, eIF5A, Enolase 2/Neuron-specific Enolase, epsilon-Sarcoglycan, FABP3/H-FABP, GDF-8/Myostatin, GDF-11/GDF-8, Integrin alpha 7, Integrin alpha 7 beta 1, Integrin beta 1/CD29, MCAM/CD146, MyoD, Myogenin, Myosin Light Chain Kinase Inhibitors, NCAM-1/CD56, and Troponin I. In some embodiments, the muscle-targeting antibody is an antibody that specifically binds a smooth muscle protein. Exemplary smooth muscle proteins include, without limitation, alpha-Smooth Muscle Actin, VE-Cadherin, Caldesmon/CALD1, Calponin 1, Desmin, Histamine H2 R, Motilin R/GPR38, Transgelin/TAGLN, and Vimentin. However, it should be appreciated that antibodies to additional targets are within the scope of this disclosure and the exemplary lists of targets provided herein are not meant to be limiting.

### **c. Antibody Features/Alterations**

**[000141]** In some embodiments, conservative mutations can be introduced into antibody sequences (e.g., CDRs or framework sequences) at positions where the residues are not likely to be involved in interacting with a target antigen (e.g., transferrin receptor), for example, as determined based on a crystal structure. In some embodiments, one, two or more mutations (e.g., amino acid substitutions) are introduced into the Fc region of a muscle-targeting antibody described herein (e.g., in a CH2 domain (residues 231-340 of human IgG1) and/or CH3 domain (residues 341-447 of human IgG1) and/or the hinge region, with numbering according to the Kabat numbering system (e.g., the EU index in Kabat)) to alter one or more functional

- 46 -

properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding and/or antigen-dependent cellular cytotoxicity.

**[000142]** 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.

**[000143]** 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 CH3 domain (residues 341-447 of human IgG1) and/or 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 region 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.

**[000144]** 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*.

**[000145]** In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant domain, or FcRn-binding fragment thereof (preferably an Fc or hinge-Fc domain fragment) to decrease the half-life of the anti-transferrin receptor antibody *in vivo*. In some embodiments, one, two or more amino acid mutations (*i.e.*, substitutions, insertions or deletions) are introduced into an IgG constant

- 47 -

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 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.

**[000146]** In some embodiments, one, two or more amino acid substitutions are introduced into an IgG constant domain Fc region to alter the effector function(s) of the anti-transferrin receptor antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in further detail in U.S. Pat. Nos. 5,624,821 and 5,648,260. In some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region domain can reduce Fc receptor binding of the circulating antibody thereby increasing tumor localization. See, *e.g.*, U.S. Pat. Nos. 5,585,097 and 8,591,886 for a description of mutations that delete or inactivate the constant domain and thereby increase tumor localization. In some embodiments, one or more amino acid substitutions may be introduced into the Fc region of an antibody described herein to remove potential glycosylation sites on Fc region, which may reduce Fc receptor binding (see, *e.g.*, Shields R L et al., (2001) *J Biol Chem* 276: 6591-604).

**[000147]** In some embodiments, one or more amino in the constant region of a muscle-targeting antibody described herein can be replaced with a different amino acid residue such that the antibody has altered Clq binding and/or 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

- 48 -

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 to increase the affinity of the antibody for an Fcγ receptor. This approach is described further in International Publication No. WO 00/42072.

**[000148]** In some embodiments, the heavy and/or 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.

**[000149]** 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.

**[000150]** As provided herein, antibodies of this disclosure may optionally comprise constant regions or parts thereof. For example, a VL domain may be attached at its C-terminal end to a light chain constant domain like Cκ or Cλ. Similarly, a VH domain or portion thereof may be attached to all or part of a heavy chain like IgA, IgD, IgE, IgG, and IgM, and any isotype subclass. Antibodies may include suitable constant regions (see, for example, Kabat et al., *Sequences of Proteins of Immunological Interest*, No. 91-3242, National Institutes of Health Publications, Bethesda, Md. (1991)). Therefore, antibodies within the scope of this may

- 49 -

disclosure include VH and VL domains, or an antigen binding portion thereof, combined with any suitable constant regions.

## ii. Muscle-Targeting Peptides

**[000151]** Some aspects of the disclosure provide muscle-targeting peptides as muscle-targeting agents. Short peptide sequences (e.g., peptide sequences of 5-20 amino acids in length) that bind to specific cell types have been described. For example, cell-targeting peptides have been described in Vines e., et al., A. “Cell-penetrating and cell-targeting peptides in drug delivery” *Biochim Biophys Acta* 2008, 1786: 126-38; Jarver P., et al., “In vivo biodistribution and efficacy of peptide mediated delivery” *Trends Pharmacol Sci* 2010; 31: 528-35; Samoylova T.I., et al., “Elucidation of muscle-binding peptides by phage display screening” *Muscle Nerve* 1999; 22: 460-6; U.S. Patent No. 6,329,501, issued on December 11, 2001, entitled “METHODS AND COMPOSITIONS FOR TARGETING COMPOUNDS TO MUSCLE”; and Samoylov A.M., et al., “Recognition of cell-specific binding of phage display derived peptides using an acoustic wave sensor.” *Biomol Eng* 2002; 18: 269-72; the entire contents of each of which are incorporated herein by reference. By designing peptides to interact with specific cell surface antigens (e.g., receptors), selectivity for a desired tissue, e.g., muscle, can be achieved. Skeletal muscle-targeting has been investigated and a range of molecular payloads are able to be delivered. These approaches may have high selectivity for muscle tissue without many of the practical disadvantages of a large antibody or viral particle. Accordingly, in some embodiments, the muscle-targeting agent is a muscle-targeting peptide that is from 4 to 50 amino acids in length. In some embodiments, the muscle-targeting peptide is 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 amino acids in length. Muscle-targeting peptides can be generated using any of several methods, such as phage display.

**[000152]** In some embodiments, a muscle-targeting peptide may bind to an internalizing cell surface receptor that is overexpressed or relatively highly expressed in muscle cells, e.g. a transferrin receptor, compared with certain other cells. In some embodiments, a muscle-targeting peptide may target, e.g., bind to, a transferrin receptor. In some embodiments, a peptide that targets a transferrin receptor may comprise a segment of a naturally occurring ligand, e.g., transferrin. In some embodiments, a peptide that targets a transferrin receptor is as described in US Patent No. 6,743,893, filed 11/30/2000, “RECEPTOR-MEDIATED UPTAKE OF PEPTIDES THAT BIND THE HUMAN TRANSFERRIN RECEPTOR”. In some

- 50 -

embodiments, a peptide that targets a transferrin receptor is as described in Kawamoto, M. et al, “A novel transferrin receptor-targeted hybrid peptide disintegrates cancer cell membrane to induce rapid killing of cancer cells.” *BMC Cancer*. 2011 Aug 18;11:359. In some embodiments, a peptide that targets a transferrin receptor is as described in US Patent No. 8,399,653, filed 5/20/2011, “TRANSFERRIN/TRANSFERRIN RECEPTOR-MEDIATED SIRNA DELIVERY”.

**[000153]** As discussed above, examples of muscle targeting peptides have been reported. For example, muscle-specific peptides were identified using phage display library presenting surface heptapeptides. As one example a peptide having the amino acid sequence ASSLNIA (SEQ ID NO: 6) bound to C2C12 murine myotubes *in vitro*, and bound to mouse muscle tissue *in vivo*. Accordingly, in some embodiments, the muscle-targeting agent comprises the amino acid sequence ASSLNIA (SEQ ID NO: 6). This peptide displayed improved specificity for binding to heart and skeletal muscle tissue after intravenous injection in mice with reduced binding to liver, kidney, and brain. Additional muscle-specific peptides have been identified using phage display. For example, a 12 amino acid peptide was identified by phage display library for muscle targeting in the context of treatment for DMD. See, Yoshida D., et al., “Targeting of salicylate to skin and muscle following topical injections in rats.” *Int J Pharm* 2002; 231: 177-84; the entire contents of which are hereby incorporated by reference. Here, a 12 amino acid peptide having the sequence SKTFNTHPQSTP (SEQ ID NO: 7) was identified and this muscle-targeting peptide showed improved binding to C2C12 cells relative to the ASSLNIA (SEQ ID NO: 6) peptide.

**[000154]** An additional method for identifying peptides selective for muscle (*e.g.*, skeletal muscle) over other cell types includes *in vitro* selection, which has been described in Ghosh D., et al., “Selection of muscle-binding peptides from context-specific peptide-presenting phage libraries for adenoviral vector targeting” *J Virol* 2005; 79: 13667-72; the entire contents of which are incorporated herein by reference. By pre-incubating a random 12-mer peptide phage display library with a mixture of non-muscle cell types, non-specific cell binders were selected out. Following rounds of selection the 12 amino acid peptide TARGEHKEEELI (SEQ ID NO: 8) appeared most frequently. Accordingly, in some embodiments, the muscle-targeting agent comprises the amino acid sequence TARGEHKEEELI (SEQ ID NO: 8).

**[000155]** A muscle-targeting agent may be an amino acid-containing molecule or peptide. A muscle-targeting peptide may correspond to a sequence of a protein that preferentially binds to a

- 51 -

protein receptor found in muscle cells. In some embodiments, a muscle-targeting peptide contains a high propensity of hydrophobic amino acids, e.g. valine, such that the peptide preferentially targets muscle cells. In some embodiments, a muscle-targeting peptide has not been previously characterized or disclosed. These peptides may be conceived of, produced, synthesized, and/or derivatized using any of several methodologies, e.g. phage displayed peptide libraries, one-bead one-compound peptide libraries, or positional scanning synthetic peptide combinatorial libraries. Exemplary methodologies have been characterized in the art and are incorporated by reference (Gray, B.P. and Brown, K.C. “Combinatorial Peptide Libraries: Mining for Cell-Binding Peptides” *Chem Rev.* 2014, 114:2, 1020–1081.; Samoylova, T.I. and Smith, B.F. “Elucidation of muscle-binding peptides by phage display screening.” *Muscle Nerve*, 1999, 22:4. 460-6.). In some embodiments, a muscle-targeting peptide has been previously disclosed (see, e.g. Writer M.J. et al. “Targeted gene delivery to human airway epithelial cells with synthetic vectors incorporating novel targeting peptides selected by phage display.” *J. Drug Targeting*. 2004;12:185; Cai, D. “BDNF-mediated enhancement of inflammation and injury in the aging heart.” *Physiol Genomics*. 2006, 24:3, 191-7.; Zhang, L. “Molecular profiling of heart endothelial cells.” *Circulation*, 2005, 112:11, 1601-11.; McGuire, M.J. et al. “In vitro selection of a peptide with high selectivity for cardiomyocytes in vivo.” *J Mol Biol*. 2004, 342:1, 171-82.). Exemplary muscle-targeting peptides comprise an amino acid sequence of the following group: CQAQGQLVC (SEQ ID NO: 9), CSERSMNFC (SEQ ID NO: 10), CPKTRRVPC (SEQ ID NO: 11), WLSEAGPVVTVRALRGTSW (SEQ ID NO: 12), ASSLNIA (SEQ ID NO: 6), CMQHSMRVC (SEQ ID NO: 13), and DDTRHWG (SEQ ID NO: 14). In some embodiments, a muscle-targeting peptide may comprise about 2-25 amino acids, about 2-20 amino acids, about 2-15 amino acids, about 2-10 amino acids, or about 2-5 amino acids. Muscle-targeting peptides may comprise naturally-occurring amino acids, e.g. cysteine, alanine, or non-naturally-occurring or modified amino acids. Non-naturally occurring amino acids include  $\beta$ -amino acids, homo-amino acids, proline derivatives, 3-substituted alanine derivatives, linear core amino acids, N-methyl amino acids, and others known in the art. In some embodiments, a muscle-targeting peptide may be linear; in other embodiments, a muscle-targeting peptide may be cyclic, e.g. bicyclic (see, e.g. Silvana, M.G. et al. *Mol. Therapy*, 2018, 26:1, 132–147.).

### iii. Muscle-Targeting Receptor Ligands

- 52 -

**[000156]** A muscle-targeting agent may be a ligand, e.g. a ligand that binds to a receptor protein. A muscle-targeting ligand may be a protein, e.g. transferrin, which binds to an internalizing cell surface receptor expressed by a muscle cell. Accordingly, in some embodiments, the muscle-targeting agent is transferrin, or a derivative thereof that binds to a transferrin receptor. A muscle-targeting ligand may alternatively be a small molecule, e.g. a lipophilic small molecule that preferentially targets muscle cells relative to other cell types. Exemplary lipophilic small molecules that may target muscle cells include compounds comprising cholesterol, cholesteryl, stearic acid, palmitic acid, oleic acid, oleyl, linolene, linoleic acid, myristic acid, sterols, dihydrotestosterone, testosterone derivatives, glycerine, alkyl chains, trityl groups, and alkoxy acids.

#### **iv. Muscle-Targeting Aptamers**

**[000157]** A muscle-targeting agent may be an aptamer, e.g. an RNA aptamer, which preferentially targets muscle cells relative to other cell types. In some embodiments, a muscle-targeting aptamer has not been previously characterized or disclosed. These aptamers may be conceived of, produced, synthesized, and/or derivatized using any of several methodologies, e.g. Systematic Evolution of Ligands by Exponential Enrichment. Exemplary methodologies have been characterized in the art and are incorporated by reference (Yan, A.C. and Levy, M. “Aptamers and aptamer targeted delivery” RNA biology, 2009, 6:3, 316-20.; Germer, K. et al. “RNA aptamers and their therapeutic and diagnostic applications.” Int. J. Biochem. Mol. Biol. 2013; 4: 27–40.). In some embodiments, a muscle-targeting aptamer has been previously disclosed (see, e.g. Phillippou, S. et al. “Selection and Identification of Skeletal-Muscle-Targeted RNA Aptamers.” Mol Ther Nucleic Acids. 2018, 10:199-214.; Thiel, W.H. et al. “Smooth Muscle Cell-targeted RNA Aptamer Inhibits Neointimal Formation.” Mol Ther. 2016, 24:4, 779-87.). Exemplary muscle-targeting aptamers include the A01B RNA aptamer and RNA Apt 14. In some embodiments, an aptamer is a nucleic acid-based aptamer, an oligonucleotide aptamer or a peptide aptamer. In some embodiments, an aptamer may be about 5-15 kDa, about 5-10 kDa, about 10-15 kDa, about 1-5 Da, about 1-3 kDa, or smaller.

#### **v. Other Muscle-Targeting Agents**

**[000158]** One strategy for targeting a muscle cell (e.g., a skeletal muscle cell) is to use a substrate of a muscle transporter protein, such as a transporter protein expressed on the sarcolemma. In some embodiments, the muscle-targeting agent is a substrate of an influx transporter that is specific to muscle tissue. In some embodiments, the influx transporter is

- 53 -

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

**[000159]** In some embodiments, the muscle-targeting agent is a substrate of an SLC superfamily of transporters. SLC transporters are either equilibrative or use proton or sodium ion gradients created across the membrane to drive transport of substrates. Exemplary SLC transporters that have high skeletal muscle expression include, without limitation, the SATT transporter (ASCT1; SLC1A4), GLUT4 transporter (SLC2A4), GLUT7 transporter (GLUT7; SLC2A7), ATRC2 transporter (CAT-2; SLC7A2), LAT3 transporter (KIAA0245; SLC7A6), PHT1 transporter (PTR4; SLC15A4), OATP-J transporter (OATP5A1; SLC21A15), OCT3 transporter (EMT; SLC22A3), OCTN2 transporter (FLJ46769; SLC22A5), ENT transporters (ENT1; SLC29A1 and ENT2; SLC29A2), PAT2 transporter (SLC36A2), and SAT2 transporter (KIAA1382; SLC38A2). These transporters can facilitate the influx of substrates into skeletal muscle, providing opportunities for muscle targeting.

**[000160]** In some embodiments, the muscle-targeting agent is a substrate of an equilibrative nucleoside transporter 2 (ENT2) transporter. Relative to other transporters, ENT2 has one of the highest mRNA expressions in skeletal muscle. While human ENT2 (hENT2) is expressed in most body organs such as brain, heart, placenta, thymus, pancreas, prostate, and kidney, it is especially abundant in skeletal muscle. Human ENT2 facilitates the uptake of its substrates depending on their concentration gradient. ENT2 plays a role in maintaining nucleoside homeostasis by transporting a wide range of purine and pyrimidine nucleobases. The hENT2 transporter has a low affinity for all nucleosides (adenosine, guanosine, uridine, thymidine, and cytidine) except for inosine. Accordingly, in some embodiments, the muscle-targeting agent is an ENT2 substrate. Exemplary ENT2 substrates include, without limitation, inosine, 2',3'-dideoxyinosine, and calofarabine. In some embodiments, any of the muscle-targeting agents provided herein are associated with a molecular payload (e.g., oligonucleotide

- 54 -

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

**[000161]** In some embodiments, the muscle-targeting agent is a substrate of an organic cation/carnitine transporter (OCTN2), which is a sodium ion-dependent, high affinity carnitine transporter. In some embodiments, the muscle-targeting agent is carnitine, mildronate, acetylcarnitine, or any derivative thereof that binds to OCTN2. In some embodiments, the carnitine, mildronate, acetylcarnitine, or derivative thereof is covalently linked to the molecular payload (e.g., oligonucleotide payload).

**[000162]** A muscle-targeting agent may be a protein that exists in at least one soluble form that targets muscle cells. In some embodiments, a muscle-targeting protein may be hemojuvelin (also known as repulsive guidance molecule C or hemochromatosis type 2 protein), a protein involved in iron overload and homeostasis. In some embodiments, hemojuvelin may be full length or a fragment, or a mutant with at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% sequence identity to a functional hemojuvelin protein. In some embodiments, a hemojuvelin mutant may be a soluble fragment, may lack a N-terminal signaling, and/or lack a C-terminal anchoring domain. In some embodiments, hemojuvelin may be annotated under GenBank RefSeq Accession Numbers NM\_001316767.1, NM\_145277.4, NM\_202004.3, NM\_213652.3, or NM\_213653.3. It should be appreciated that a hemojuvelin may be of human, non-human primate, or rodent origin.

## **B. Molecular Payloads**

**[000163]** 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. In some embodiments, a molecular payload is linked to, or otherwise associated with a muscle-targeting agent. In some embodiments, such molecular payloads are capable of targeting to a muscle cell, e.g., via specifically binding to a nucleic acid or protein in the muscle cell following delivery to the muscle cell by an associated muscle-targeting agent. It should be appreciated that various types of muscle-targeting agents may be used in accordance with the disclosure. For example, the molecular payload may comprise, or consist of, an oligonucleotide (e.g., antisense oligonucleotide), a peptide (e.g., a peptide that binds a nucleic acid or protein associated with disease in a muscle cell), a protein (e.g., a protein that binds a nucleic acid or protein associated with disease in a muscle cell), or a small molecule (e.g., a

- 55 -

small molecule that modulates the function of a nucleic acid or protein associated with disease in a muscle cell). In some embodiments, the molecular payload is an oligonucleotide that comprises a strand having a region of complementarity to FXN (e.g., a GAA repeat). 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**

**[000164]** 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.

**[000165]** Examples of oligonucleotides useful for targeting FXN and/or otherwise compensating for frataxin deficiency are provided in Li, L. et al “Activating frataxin expression by repeat-targeted nucleic acids” Nat. Comm. 2016, 7:10606.; WO 2016/094374, published 6/16/2016, “Compositions and methods for treatment of Friedreich's ataxia.”; WO 2015/020993, published 2/12/2015, “RNAi COMPOSITIONS AND METHODS FOR TREATMENT OF FRIEDREICH'S ATAXIA”; WO 2017/186815, published 11/2/2017, “Antisense oligonucleotides for enhanced expression of frataxin”; WO 2008/018795, published 2/14/2008, “Methods and means for treating DNA repeat instability associated genetic disorders”; US Patent Application 2018/0028557, published 2/1/2018, “Hybrid oligonucleotides and uses thereof”; WO 2015/023975, published 2/19/2015, “Compositions and methods for modulating RNA”; WO 2015/023939, published 2/19/2015, “Compositions and methods for modulating expression of frataxin”; US Patent Application 2017/0281643, published 10/5/2017, “Compounds and methods for modulating frataxin expression”; Li L. et al., “Activating frataxin

- 56 -

expression by repeat-targeted nucleic acids” Nature Communications, Published 4 Feb 2016; and Li L. et al. “Activation of Frataxin Protein Expression by Antisense Oligonucleotides Targeting the Mutant Expanded Repeat” Nucleic Acid Ther. 2018 Feb;28(1):23-33., the contents of each of which are incorporated herein in their entireties.

**[000166]** In some embodiments, an oligonucleotide payload is configured (e.g., as a gapmer or RNAi oligonucleotide) for inhibiting expression of a natural antisense transcript that inhibits FXN expression, e.g., as disclosed in US Patent No. 9,593,330, filed 6/9/2011, “Treatment of frataxin (FXN) related diseases by inhibition of natural antisense transcript to FXN”, the contents of which are incorporated herein by reference in its entirety.

**[000167]** Examples of oligonucleotides for promoting FXN gene editing include WO 2016/094845, published 6/16/2016, “Compositions and methods for editing nucleic acids in cells utilizing oligonucleotides”; WO 2015/089354, published 6/18/2015, “Compositions and methods of use of CRISPR-Cas systems in nucleotide repeat disorders”; WO 2015/139139, published 9/24/2015, “CRISPR-based methods and products for increasing frataxin levels and uses thereof”; and WO 2018/002783, published 1/4/2018, “Materials and methods for treatment of Friedreich’s ataxia and other related disorders”, the contents of each of which are incorporated herein in their entireties.

**[000168]** Examples of oligonucleotides for promoting FXN gene expression through targeting of non-FXN genes, e.g. epigenetic regulators of FXN, include WO 2015/023938, published 2/19/2015, “Epigenetic regulators of frataxin”, the contents of which are incorporated herein in its entirety.

**[000169]** In some embodiments, oligonucleotides may have a region of complementarity to a sequence set forth as: a FXN gene from humans (Gene ID 2395; NC\_000009.12) and/or a FXN gene from mice (Gene ID 14297; NC\_000085.6). In some embodiments, the oligonucleotide may have region of complementarity to a mutant form of FXN, for example as reported in e.g., Montermini, L. et al. “The Friedreich’s ataxia GAA triplet repeat: premutation and normal alleles.” Hum. Molec. Genet., 1997, 6: 1261-1266.; Filla, A. et al. “The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich’s ataxia.” Am. J. Hum. Genet. 1996, 59: 554-560.; Pandolfo, M. Friedreich’s ataxia: the clinical picture. J. Neurol. 2009, 256, 3–8.; the contents of each of which are incorporated herein by reference in their entireties.

#### **a. Oligonucleotide Size/Sequence**

- 57 -

**[000170]** 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.

**[000171]** 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.

**[000172]** 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.

- 58 -

**b. Oligonucleotide Modifications:**

**[000173]** The oligonucleotides described herein may be modified, e.g., comprise a modified sugar moiety, a modified internucleoside linkage, a modified nucleotide and/or 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.

**[000174]** 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.

**[000175]** 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 Nucleotides**

- 59 -

**[000176]** In some embodiments, an oligonucleotide include a 2'-modified nucleotide, e.g., a 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-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).

**[000177]** In some embodiments, an oligonucleotide can include at least one 2'-O-methyl-modified nucleotide, and in some embodiments, all of the nucleotides include a 2'-O-methyl modification. In some embodiments, an oligonucleotide comprises modified nucleotides 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. In some embodiments, the oligonucleotides are "locked," e.g., comprise modified nucleotides in which the ribose ring is "locked" by a methylene bridge connecting the 2'-O atom and the 4'-C atom. 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.

**[000178]** Other modifications that may be used in the oligonucleotides disclosed herein include ethylene-bridged nucleic acids (ENAs). ENAs include, but are not limited to, 2'-O,4'-C-ethylene-bridged nucleic acids. 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.

**[000179]** In some embodiments, the oligonucleotide may comprise a bridged nucleotide, such as a locked nucleic acid (LNA) nucleotide, a constrained ethyl (cEt) nucleotide, or an ethylene bridged nucleic acid (ENA) nucleotide. In some embodiments, the oligonucleotide comprises a modified nucleotide 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*

- 60 -

*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.

**[000180]** In some embodiments, the oligonucleotide comprises at least one nucleotide modified at the 2' position of the sugar, preferably a 2'-O-alkyl, 2'-O-alkyl-O-alkyl or 2'-fluoro-modified nucleotide. In other preferred embodiments, RNA modifications include 2'-fluoro, 2'-amino and 2' O-methyl modifications on the ribose of pyrimidines, abasic residues or an inverted base at the 3' end of the RNA.

**[000181]** In some embodiments, the oligonucleotide may have at least one modified nucleotide that results in an increase in T<sub>m</sub> 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 nucleotide . The oligonucleotide may have a plurality of modified nucleotides that result in a total increase in T<sub>m</sub> 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 nucleotide .

**[000182]** The oligonucleotide may comprise alternating nucleotides of different kinds. For example, an oligonucleotide may comprise alternating deoxyribonucleotides or ribonucleotides and 2'-fluoro-deoxyribonucleotides. An oligonucleotide may comprise alternating deoxyribonucleotides or ribonucleotides and 2'-O-methyl nucleotides. An oligonucleotide may comprise alternating 2'-fluoro nucleotides and 2'-O-methyl nucleotides. An oligonucleotide may comprise alternating bridged nucleotides and 2'-fluoro or 2'-O-methyl nucleotides.

#### **d. Internucleotide Linkages / Backbones**

**[000183]** In some embodiments, oligonucleotide may contain a phosphorothioate or other modified internucleotide 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

- 61 -

internucleotide linkages at the first, second, and/or third internucleoside linkage at the 5' or 3' end of the nucleotide sequence.

**[000184]** 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.

**[000185]** In some embodiments, oligonucleotides may have heteroatom backbones, such as methylene(methylimino) or MMI backbones; amide backbones (see De Mesmaeker et al. *Ace. Chem. Res.* 1995, 28:366-374); morpholino backbones (see Summerton and Weller, U.S. Pat. No. 5,034,506); or peptide nucleic acid (PNA) backbones (wherein the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleotides being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone, see Nielsen et al., *Science* 1991, 254, 1497).

#### **e. Stereospecific Oligonucleotides**

**[000186]** In some embodiments, internucleotidic phosphorus atoms of oligonucleotides are chiral, and the properties of the oligonucleotides are 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 are provided that comprise nucleoside units that are joined together by either substantially all Sp or substantially all Rp phosphorothioate intersugar linkages. 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

- 62 -

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**

[000187] In some embodiments, the oligonucleotide may be a morpholino-based compounds. Morpholino-based oligomeric compounds are described in Dwaine A. Braasch and David R. Corey, *Biochemistry*, 2002, 41(14), 4503-4510; *Genesis*, volume 30, issue 3, 2001; Heasman, J., *Dev. Biol.*, 2002, 243, 209-214; Nasevicius et al., *Nat. Genet.*, 2000, 26, 216-220; Lacerra et al., *Proc. Natl. Acad. Sci.*, 2000, 97, 9591-9596; and U.S. Pat. No. 5,034,506, issued Jul. 23, 1991. In some embodiments, the morpholino-based oligomeric compound is a phosphorodiamidate morpholino oligomer (PMO) (e.g., as described in Iverson, *Curr. Opin. Mol. Ther.*, 3:235-238, 2001; and Wang et al., *J. Gene Med.*, 12:354-364, 2010; the disclosures of which are incorporated herein by reference in their entirety).

**g. Peptide Nucleic Acids (PNAs)**

[000188] 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**

- 63 -

**[000189]** In some embodiments, the oligonucleotide 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, the Y region is a contiguous stretch of nucleotides, e.g., a region of at least 6 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 nucleotides, e.g., one to six modified nucleotides. Examples of modified nucleotides include, but are not limited to, 2' MOE or 2'OMe or Locked Nucleic Acid bases (LNA). The flanking sequences X and Z may be of one to twenty nucleotides, one to eight nucleotides or one to five nucleotides in length, in some embodiments. The flanking sequences X and Z may be of similar length or of dissimilar lengths. The gap-segment Y may be a nucleotide sequence of five to twenty nucleotides, size to twelve nucleotides or six to ten nucleotides in length, in some embodiments.

**[000190]** In some embodiments, the gap region of the gapmer oligonucleotides may contain modified nucleotides known to be acceptable for efficient RNase H action in addition to DNA nucleotides, such as C4'-substituted nucleotides, acyclic nucleotides, and arabino-configured nucleotides. In some embodiments, the gap region comprises one or more unmodified internucleosides. In some embodiments, one or both flanking regions each independently comprise one or more phosphorothioate internucleoside linkages (e.g., phosphorothioate internucleoside linkages or other linkages) between at least two, at least three, at least four, at least five or more nucleotides. In some embodiments, the gap region and two flanking regions each independently comprise modified internucleoside linkages (e.g., phosphorothioate internucleoside linkages or other linkages) between at least two, at least three, at least four, at least five or more nucleotides.

**[000191]** 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; 7,432,250; and 7,683,036; U.S. patent publication Nos. US20090286969, US20100197762, and US20110112170; and PCT publication Nos. WO2008049085 and WO2009090182, each of which is herein incorporated by reference in its entirety.

- 64 -

**i. Mixmers**

**[000192]** 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 nucleotides or comprise two different types of non-naturally occurring nucleotides 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.

**[000193]** In some embodiments, the mixmer comprises or consists of a repeating pattern of nucleotide analogues and naturally occurring nucleotides, or one type of nucleotide analogue and a second type of nucleotide analogue. However, a mixmer need not comprise a repeating pattern and may instead comprise any arrangement of modified nucleotides and naturally occurring nucleotides or any arrangement of one type of modified nucleotide and a second type of modified nucleotide. The repeating pattern, may, for instance be every second or every third nucleotide is a modified nucleotide, such as LNA, and the remaining nucleotides are naturally occurring nucleotides, such as DNA, or are a 2' substituted nucleotide analogue such as 2'MOE or 2' fluoro analogues, or any other modified nucleotide described herein. It is recognized that the repeating pattern of modified nucleotide, such as LNA units, may be combined with modified nucleotide at fixed positions—e.g. at the 5' or 3' termini.

**[000194]** 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 nucleotides, such as DNA nucleotides. In some embodiments, the mixmer comprises at least a region consisting of at least two consecutive modified nucleotide, such as at least two consecutive LNAs. In some embodiments, the mixmer comprises at least a region consisting of at least three consecutive modified nucleotide units, such as at least three consecutive LNAs.

**[000195]** 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 nucleotide analogues, such as LNAs. In some embodiments, LNA units may be replaced with other nucleotide analogues, such as those referred to herein.

- 65 -

**[000196]** Mixmers may be designed to comprise a mixture of affinity enhancing modified nucleotides, such as in non-limiting example LNA nucleotides and 2'-O-methyl nucleotides. 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 nucleotides.

**[000197]** 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.

**[000198]** In some embodiments, a mixmer comprises one or more morpholino nucleotides. For example, in some embodiments, a mixmer may comprise morpholino nucleotides mixed (e.g., in an alternating manner) with one or more other nucleotides (e.g., DNA, RNA nucleotides) or modified nucleotides (e.g., LNA, 2'-O-Methyl nucleotides).

**[000199]** 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)**

**[000200]** 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.

**[000201]** 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

- 66 -

Publication Number WO 2004/016735; and U.S. Patent Publication Nos. 2004/0077574 and 2008/0081791).

**[000202]** The siRNA molecule can be double stranded (i.e. a dsRNA molecule comprising an antisense strand and a complementary sense strand) 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.

**[000203]** Double-stranded siRNA may comprise 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 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 the 5' end of either or both strands). A spacer sequence is may be an unrelated nucleotide sequence that is situated between two complementary nucleotide sequence regions which, when annealed into a double-stranded nucleic acid, comprise a shRNA.

**[000204]** 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

- 67 -

nucleotides, whereas when the siRNA is a shRNA or circular molecule, the length can vary from about 40 nucleotides to about 100 nucleotides.

**[000205]** 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.

#### **k. microRNA (miRNAs)**

**[000206]** 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.

**[000207]** As used herein, miRNAs including pri-miRNA, pre-miRNA, mature miRNA or fragments of variants thereof that retain the biological activity of mature miRNA. In one embodiment, the size range of the miRNA can be from 21 nucleotides to 170 nucleotides. In one embodiment the size range of the miRNA is from 70 to 170 nucleotides in length. In another embodiment, mature miRNAs of from 21 to 25 nucleotides in length can be used.

#### **l. Aptamers**

**[000208]** 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 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

- 68 -

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**

**[000209]** 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.

**[000210]** 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.

**[000211]** 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-

- 69 -

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.

**[000212]** 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 may be synthesized conventionally, for example, by using RNA polymerases such as T7 or SP6.

#### **n. Guide Nucleic Acids**

**[000213]** 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.

**[000214]** 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

- 70 -

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.

**[000215]** 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., Najjar 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**

**[000216]** 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 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).

**[000217]** 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.

- 71 -

**[000218]** In some embodiments, a multimer comprises 2 or more oligonucleotides linked end-to-end (in a linear arrangement). In some embodiments, a multimer comprises 2 or more oligonucleotides linked end-to-end via a oligonucleotide based linker (e.g., poly-dT linker, an abasic linker). In some embodiments, a multimer comprises a 5' end of one oligonucleotide linked to a 3' end of another oligonucleotide. In some embodiments, a multimer comprises a 3' end of one oligonucleotide linked to a 3' end of another oligonucleotide. In some embodiments, a multimer comprises a 5' end of one oligonucleotide linked to a 5' end of another oligonucleotide. Still, in some embodiments, multimers can comprise a branched structure comprising multiple oligonucleotides linked together by a branching linker.

**[000219]** Further examples of multimers that may be used in the complexes provided herein are disclosed, for example, in US Patent Application Number 2015/0315588 A1, entitled *Methods of delivering multiple targeting oligonucleotides to a cell using cleavable linkers*, which was published on November 5, 2015; US Patent Application Number 2015/0247141 A1, entitled *Multimeric Oligonucleotide Compounds*, which was published on September 3, 2015, US Patent Application Number US 2011/0158937 A1, entitled *Immunostimulatory Oligonucleotide Multimers*, which was published on June 30, 2011; and US Patent Number 5,693,773, entitled *Triplex-Forming Antisense Oligonucleotides Having Abasic Linkers Targeting Nucleic Acids Comprising Mixed Sequences Of Purines And Pyrimidines*, which issued on December 2, 1997, the contents of each of which are incorporated herein by reference in their entireties.

## **ii. Small Molecules:**

**[000220]** Any suitable small molecule may be used as a molecular payload, as described herein. In some embodiments, the small molecule is as described in Herman D. et al. "Histone deacetylase inhibitors reverse gene silencing in Friedreich's ataxia." *Nat Chem Biol.* 2006;2:551-558. In some embodiments, the small molecule is as described in Rai, M. et al. "HDAC inhibitors correct frataxin deficiency in a Friedreich's ataxia mouse model." *PLoS One.* 2008 Apr 9; 3(4):e1958. Further examples of small molecule payloads are provided in Richardson, T.E. et al, "Therapeutic strategies in Friedreich's Ataxia", *Brain Res.* 2013 Jun 13; 1514: 91-97; Zeier Z et al. "Bromodomain inhibitors regulate the C9ORF72 locus in ALS" *Exp Neurol.* 2015 Sep;271:241-50.; and Gottesfeld J.M. "Small molecules affecting transcription in Friedreich's ataxia." *Pharmacol Ther.* 2007 Nov;116(2):236-48. For example, in some embodiments, the small molecule is an inhibitor of a histone deacetylase, e.g., BML-210 and

- 72 -

compound 106. In some embodiments, the small molecule is 17 $\beta$ -Estradiol or methylene blue. In some embodiments, the small molecule targets, *e.g.*, binds to, a disease-associated-repeat and/or R-loop. In some embodiments, the small molecule is as described in WO 2004/003565, published 1/8/2004, “A screening method and compounds for treating Friedreich’s ataxia”. In some embodiments, the small molecule is a Glutathione peroxidase mimetic.

### iii. Peptides/Proteins

**[000221]** Any suitable peptide or protein may be used as a molecular payload, as described herein. In some embodiments, a protein is an enzyme. These peptides or proteins may be produced, synthesized, and/or 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.).

**[000222]** In some embodiments, the peptide is as described in US Patent No. 8,815,230, filed 8/30/2010, “Methods for treating Friedreich’s ataxia with interferon gamma”. In some embodiments, the peptide is as described in Britti, E. et al. “Frataxin-deficient neurons and mice models of Friedreich’s ataxia are improved by TAT-MTScs-FXN treatment.” *J Cell Mol Med.* 2018 Feb;22(2):834-848. In some embodiments, the peptide is as described in Zhao, H. et al., “Peptide SS-31 upregulates frataxin expression and improves the quality of mitochondria: implications in the treatment of Friedreich’s ataxia”, *Sci Rep.* 2017 Aug 29;7(1):9840. In some embodiments, the peptide is as described in Vyas, P.M. et al. “A TAT-frataxin fusion protein increases lifespan and cardiac function in a conditional Friedreich’s ataxia mouse model”, *Hum Mol Genet.* 2012 Mar 15;21(6):1230-47. In some embodiments, the peptide or protein may target, *e.g.*, bind to, a disease-associated repeat, *e.g.* a GAA repeat expansion.

**[000223]** In some embodiments, the peptide or protein may comprise about 2-25 amino acids, about 2-20 amino acids, about 2-15 amino acids, about 2-10 amino acids, or about 2-5 amino acids. The peptide or protein may comprise naturally-occurring amino acids, *e.g.* cysteine, alanine, or non-naturally-occurring or modified amino acids. Non-naturally occurring amino acids include  $\beta$ -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

- 73 -

some embodiments, the peptide may be linear; in other embodiments, the peptide may be cyclic, e.g. bicyclic.

#### iv. Nucleic Acid Constructs

**[000224]** 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 leads to increased expression of frataxin. 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 frataxin. In some embodiments, the gene expression constructs encodes a protein that inhibit the function of epigenetic regulators that negatively regulate the expression of FXN, *e.g.* histone deacetylases. In some embodiments, the gene expression construct encodes a protein that binds to a disease-associated-repeat expansion of a GAA trinucleotide. In some embodiments, the gene expression construct encodes a protein that leads to a reduction in the expression of a epigenetic regulators that negatively regulate the expression of FXN, *e.g.* histone deacetylases. In some embodiments, the gene expression construct encodes a gene editing enzyme. In some embodiments, the gene expression construct encodes erythropoietin (see, *e.g.* Miller, J.L. et al, “Erythropoietin and small molecule agonists of the tissue-protective erythropoietin receptor increase FXN expression in neuronal cells in vitro and in FXN-deficient KIKO mice in vivo”, *Neuropharmacology*. 2017 Sep 1;123:34-45.). In some embodiments, the gene expression construct encodes interferon gamma (see, *e.g.* US Patent No. 8,815,230, filed 8/30/2010, “Methods for treating Friedreich's ataxia with interferon gamma”).

**[000225]** 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

- 74 -

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.

### C. Linkers

[000226] Complexes described herein generally comprise a linker that connects a muscle-targeting agent 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 a muscle-targeting agent to a molecular payload. However, in some embodiments, a linker may connect a muscle-targeting agent 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 muscle-targeting agent 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.).

[000227] A precursor to a linker typically will contain two different reactive species that allow for attachment to both the muscle-targeting agent and a molecular payload. In some embodiments, the two different reactive species may be a nucleophile and/or an electrophile. In some embodiments, a linker is connected to a muscle-targeting agent via conjugation to a lysine residue or a cysteine residue of the muscle-targeting agent. In some embodiments, a linker is connected to a cysteine residue of a muscle-targeting agent 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 a muscle-targeting agent or thiol functionalized molecular payload via a 3-arylpropionitrile functional group. In some embodiments, a linker is connected to a muscle-targeting agent and/or a molecular payload via an amide bond, a hydrazide, a triazole, a thioether or a disulfide bond.

#### i. Cleavable Linkers

- 75 -

**[000228]** 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.

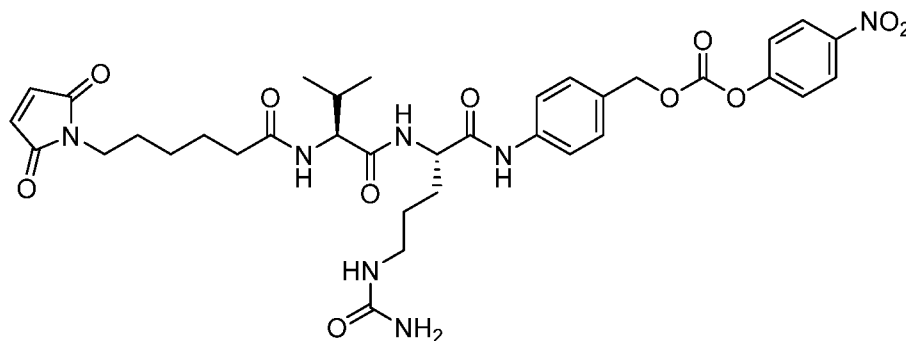
**[000229]** Protease-sensitive linkers are cleavable by protease enzymatic activity. These linkers typically comprise peptide sequences and may be 2-10 amino acids, about 2-5 amino acids, about 5-10 amino acids, about 10 amino acids, about 5 amino acids, about 3 amino acids, or about 2 amino acids in 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  $\beta$ -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 an endosomal protease.

**[000230]** 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.

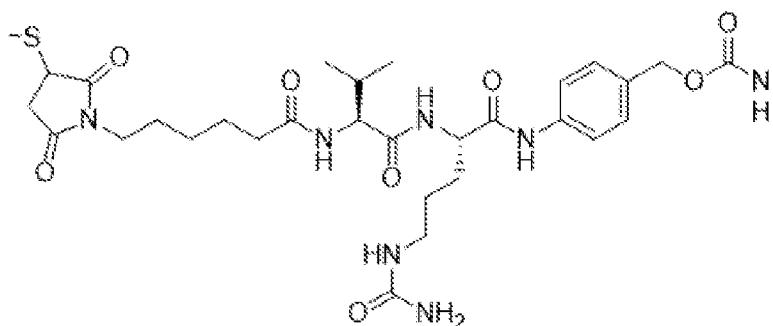
**[000231]** In some embodiments, a glutathione-sensitive linker comprises a disulfide moiety. In some embodiments, a glutathione-sensitive linker is cleaved by an disulfide exchange reaction with a glutathione species inside a cell. In some embodiments, the disulfide moiety further comprises at least one amino acid, e.g. a cysteine residue.

**[000232]** 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:

- 76 -



[000233] In some embodiments, after conjugation, the val-cit linker has a structure of:



## ii. Non-Cleavable Linkers

[000234] 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 an alkoxy-amine linker. In some embodiments, sortase-mediated ligation will be utilized to covalently link a muscle-targeting agent comprising a LPXT sequence to a molecular payload comprising a (G)<sub>n</sub> 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.).

[000235] In some embodiments, a linker may comprise a substituted alkylene, an optionally substituted alkenylene, an optionally substituted alkynylene, an optionally substituted

- 77 -

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

[000236] In some embodiments, a linker is connected to a muscle-targeting agent and/or molecular payload via a phosphate, thioether, ether, carbon-carbon, 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 muscle-targeting agent, e.g. an antibody, through a lysine or cysteine residue present on the muscle-targeting agent

[000237] In some embodiments, a linker is connected to a muscle-targeting agent and/or 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 muscle-targeting agent, 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  $\beta(1,4)$ -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 muscle-targeting agent, 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

- 78 -

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  $\beta(1,4)$ -N-Acetylgalactosaminyltransferase*”.

**[000238]** In some embodiments, a linker further comprises a spacer, *e.g.*, a polyethylene glycol spacer or an acyl/carbomoyl sulfamide spacer, *e.g.*, a HydraSpace<sup>TM</sup> 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.

**[000239]** In some embodiments, a linker is connected to a muscle-targeting agent and/or 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 muscle-targeting agent, molecular payload, or the linker. In some embodiments a linker is connected to a muscle-targeting agent and/or molecular payload by other pericyclic reactions, *e.g.* ene reaction. In some embodiments, a linker is connected to a muscle-targeting agent and/or molecular payload by an amide, thioamide, or sulfonamide bond reaction. In some embodiments, a linker is connected to a muscle-targeting agent and/or molecular payload by a condensation reaction to form an oxime, hydrazone, or semicarbazide group existing between the linker and the muscle-targeting agent and/or molecular payload.

**[000240]** In some embodiments, a linker is connected to a muscle-targeting agent and/or molecular payload by a conjugate addition reactions 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 a muscle-targeting agent or molecular payload prior to a reaction between a linker and a muscle-targeting agent or molecular payload. In some embodiments, an electrophile may exist on a linker and a nucleophile may exist on a muscle-targeting agent or molecular payload prior to a reaction between a linker and a muscle-targeting agent or molecular payload. In some embodiments, an electrophile may be an azide, 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 an activated sulfur center. In some embodiments, a nucleophile may be an optionally substituted alkene, an optionally substituted alkyne, an optionally substituted

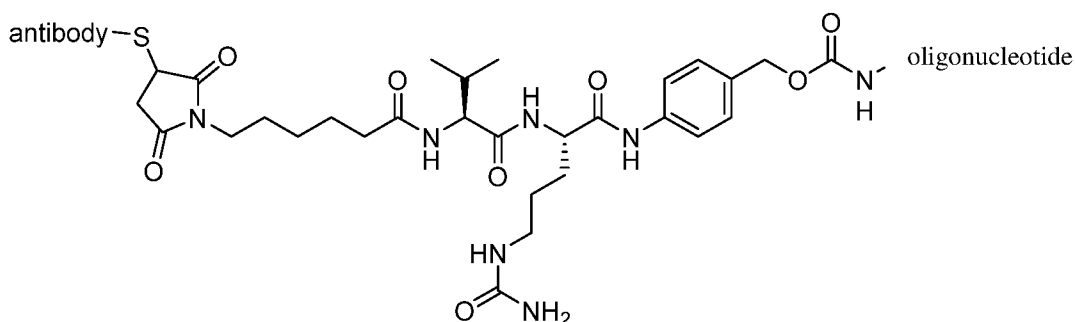
- 79 -

aryl, an optionally substituted heterocyclyl, a hydroxyl group, an amino group, an alkylamino group, an anilido group, or a thiol group.

#### D. Examples of Antibody-Molecular Payload Complexes

**[000241]** Other aspects of the present disclosure provide complexes comprising any one the muscle targeting agent (e.g., a transferrin receptor antibodies) described herein covalently linked to any of the molecular payloads (e.g., an oligonucleotide) described herein. In some embodiments, the muscle targeting agent (e.g., a transferrin receptor antibody) 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, 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 antibody via a thiol-reactive linkage (e.g., via a cysteine in the antibody).

**[000242]** An exemplary structure of a complex comprising a transferrin receptor antibody covalently linked to an oligonucleotide via a Val-cit linker is provided below:



wherein the linker is linked to the 5' end, the 3' end, or internally of the oligonucleotide, and wherein the linker is linked to the antibody via a thiol-reactive linkage (e.g., via a cysteine in the antibody).

**[000243]** It should be appreciated that antibodies can be linked to oligonucleotides with different stoichiometries, a property that may be referred to as a drug to antibody ratios (DAR) with the “drug” being the oligonucleotide. In some embodiments, one oligonucleotide is linked to an antibody (DAR = 1). In some embodiments, two oligonucleotides are linked to an antibody (DAR = 2). In some embodiments, three oligonucleotides are linked to an antibody (DAR = 3). In some embodiments, four oligonucleotides 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 oligonucleotides to different sites on an antibody and/or by conjugating multimers to one or more sites on

- 80 -

antibody. For example, a DAR of 2 may be achieved by conjugating a single oligonucleotide to two different sites on an antibody or by conjugating a dimer oligonucleotide to a single site of an antibody.

**[000244]** In some embodiments, the complex described herein comprises a transferrin receptor antibody (e.g., an antibody or any variant thereof as described herein) covalently linked to an oligonucleotide. In some embodiments, the complex described herein comprises a transferrin receptor antibody (e.g., an antibody or any variant thereof as described herein) covalently linked to an oligonucleotide via a linker (e.g., a Val-cit linker). In some embodiments, the linker (e.g., a Val-cit linker) is linked to the 5' end, the 3' end, or internally of the oligonucleotide. In some embodiments, the linker (e.g., a Val-cit linker) is linked to the antibody (e.g., an antibody or any variant thereof as described herein) via a thiol-reactive linkage (e.g., via a cysteine in the antibody).

**[000245]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide, wherein the transferrin receptor 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 1.1; 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 1.1.

**[000246]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide, wherein the transferrin receptor antibody comprises a VH having the amino acid sequence of SEQ ID NO: 33 and a VL having the amino acid sequence of SEQ ID NO: 34. In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide, wherein the transferrin receptor antibody comprises a VH having the amino acid sequence of SEQ ID NO: 35 and a VL having the amino acid sequence of SEQ ID NO: 36.

**[000247]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide, wherein the transferrin receptor antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO: 39 and a light chain having the amino acid sequence of SEQ ID NO: 40. In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide, wherein the transferrin receptor antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO: 41 and a light chain having the amino acid sequence of SEQ ID NO: 42.

- 81 -

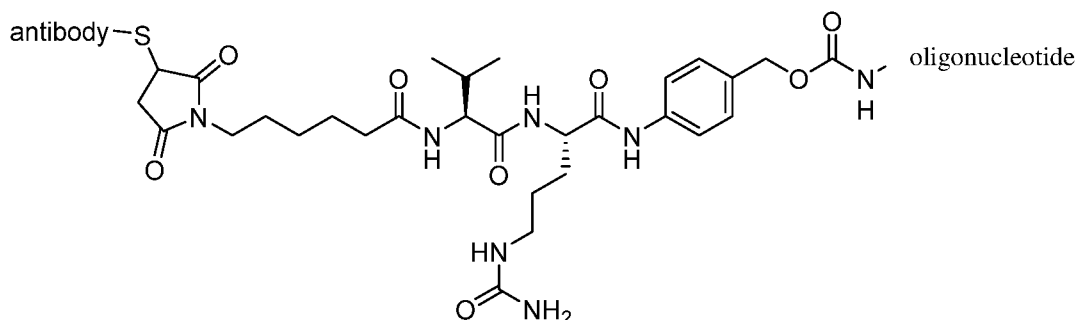
**[000248]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a linker (e.g., a Val-cit linker), wherein the transferrin receptor antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 that are the same as the CDR-H1, CDR-H2, and CDR-H3 shown in Table 1.1; 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 1.1.

**[000249]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a linker (e.g., a Val-cit linker), wherein the transferrin receptor antibody comprises a VH having the amino acid sequence of SEQ ID NO: 33 and a VL having the amino acid sequence of SEQ ID NO: 34. In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a linker (e.g., a Val-cit linker), wherein the transferrin receptor antibody comprises a VH having the amino acid sequence of SEQ ID NO: 35 and a VL having the amino acid sequence of SEQ ID NO: 36.

**[000250]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a linker (e.g., a Val-cit linker), wherein the transferrin receptor antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO: 39 and a light chain having the amino acid sequence of SEQ ID NO: 40. In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a linker (e.g., a Val-cit linker), wherein the transferrin receptor antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO: 41 and a light chain having the amino acid sequence of SEQ ID NO: 42.

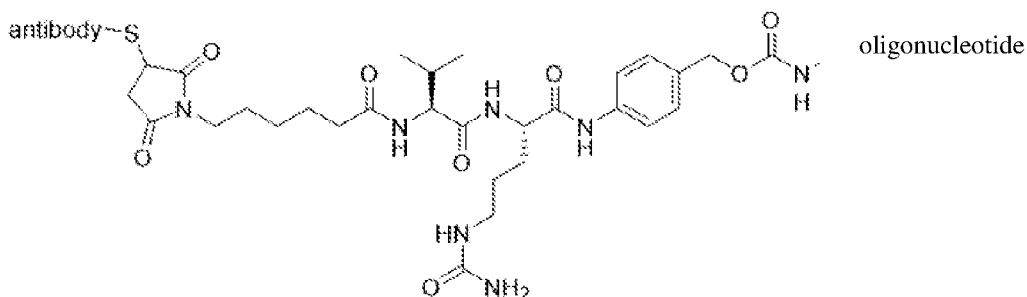
**[000251]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a Val-cit linker, wherein the transferrin receptor antibody comprises a CDR-H1, a CDR-H2, and a CDR-H3 that are the same as the CDR-H1, CDR-H2, and CDR-H3 shown in Table 1.1; 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 1.1, and wherein the complex comprises the structure of:

- 82 -



wherein the linker Val-cit linker is linked to the 5' end, the 3' end, or internally of the oligonucleotide, and wherein the Val-cit linker is linked to the antibody (e.g., an antibody or any variant thereof as described herein) via a thiol-reactive linkage (e.g., via a cysteine in the antibody).

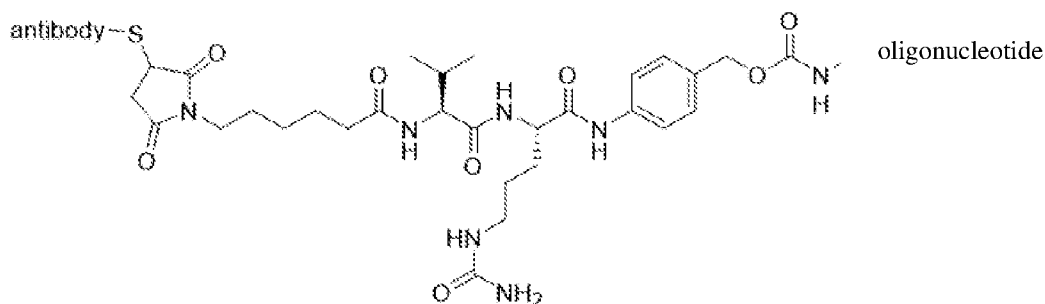
**[000252]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a Val-cit linker, wherein the transferrin receptor antibody comprises a VH having the amino acid sequence of SEQ ID NO: 33 and a VL having the amino acid sequence of SEQ ID NO: 34, and wherein the complex comprises the structure of:



wherein the linker Val-cit linker is linked to the 5' end, the 3' end, or internally of the oligonucleotide, and wherein the Val-cit linker is linked to the antibody (e.g., an antibody or any variant thereof as described herein) via a thiol-reactive linkage (e.g., via a cysteine in the antibody).

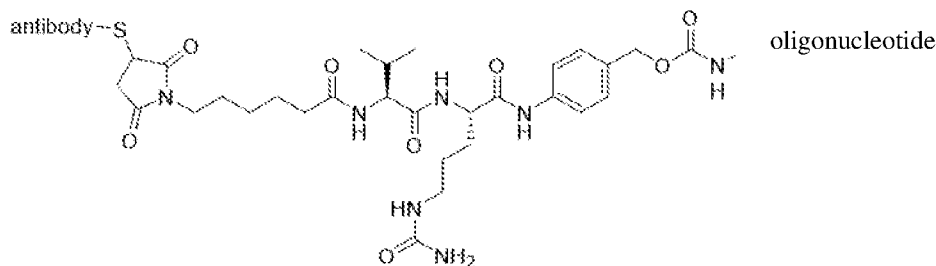
**[000253]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a Val-cit linker, wherein the transferrin receptor antibody comprises a VH having the amino acid sequence of SEQ ID NO: 35 and a VL having the amino acid sequence of SEQ ID NO: 36, and wherein the complex comprises the structure of:

- 83 -



wherein the linker Val-cit linker is linked to the 5' end, the 3' end, or internally of the oligonucleotide, and wherein the Val-cit linker is linked to the antibody (e.g., an antibody or any variant thereof as described herein) via a thiol-reactive linkage (e.g., via a cysteine in the antibody).

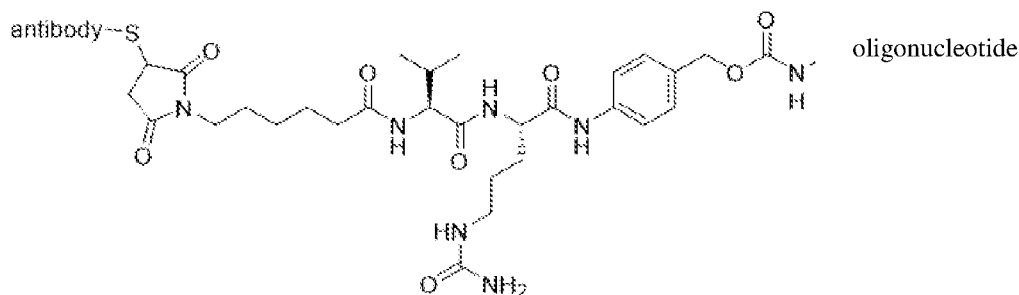
**[000254]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a Val-cit linker, wherein the transferrin receptor antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO: 39 and a light chain having the amino acid sequence of SEQ ID NO: 40, and wherein the complex comprises the structure of:



wherein the linker Val-cit linker is linked to the 5' end, the 3' end, or internally of an oligonucleotide, and wherein the Val-cit linker is linked to the antibody (e.g., an antibody or any variant thereof as described herein) via a thiol-reactive linkage (e.g., via a cysteine in the antibody).

**[000255]** In some embodiments, the complex described herein comprises a transferrin receptor antibody covalently linked to an oligonucleotide via a Val-cit linker, wherein the transferrin receptor antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO: 41 and a light chain having the amino acid sequence of SEQ ID NO: 42, and wherein the complex comprises the structure of:

- 84 -



wherein the linker Val-cit linker is linked to the 5' end, the 3' end, or internally of an oligonucleotide, and wherein the Val-cit linker is linked to the antibody (e.g., an antibody or any variant thereof as described herein) via a thiol-reactive linkage (e.g., via a cysteine in the antibody).

### III. Formulations

**[000256]** Complexes provided herein may be formulated in any suitable manner.

Generally, complexes provided herein are formulated in a manner suitable for pharmaceutical use. For example, complexes can be delivered to a subject using a formulation that minimizes degradation, facilitates delivery and/or uptake, or provides another beneficial property to the complexes in the formulation. In some embodiments, provided herein are compositions comprising complexes and pharmaceutically acceptable carriers. Such compositions can be suitably formulated such that when administered to a subject, either into the immediate environment of a target cell or systemically, a sufficient amount of the complexes enter target muscle cells. In some embodiments, complexes are formulated in buffer solutions such as phosphate-buffered saline solutions, liposomes, micellar structures, and capsids.

**[000257]** It should be appreciated that, in some embodiments, compositions may include separately one or more components of complexes provided herein (e.g., muscle-targeting agents, linkers, molecular payloads, or precursor molecules of any one of them).

**[000258]** In some embodiments, complexes are formulated in water or in an aqueous solution (e.g., water with pH adjustments). In some embodiments, complexes are formulated in basic buffered aqueous solutions (e.g., PBS). In some embodiments, formulations as disclosed herein comprise an excipient. In some embodiments, an excipient confers to a composition improved stability, improved absorption, improved solubility and/or therapeutic enhancement of the active ingredient. In some embodiments, an excipient is a buffering agent (e.g., sodium

- 85 -

citrate, sodium phosphate, a tris base, or sodium hydroxide) or a vehicle (*e.g.*, a buffered solution, petrolatum, dimethyl sulfoxide, or mineral oil).

**[000259]** 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).

**[000260]** 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.

**[000261]** 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 a complexes in a required amount in a selected solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

**[000262]** In some embodiments, a composition may contain at least about 0.1% of the a complex, or component thereof, or more, although the percentage of the active ingredient(s) may be between about 1% and about 80% or more of the weight or volume of the total composition. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

#### **IV. Methods of Use / Treatment**

- 86 -

**[000263]** Complexes comprising a muscle-targeting agent covalently to a molecular payload as described herein are effective in treating Friedreich's ataxia. In some embodiments, complexes are effective in treating early onset or late onset Friedreich's ataxia. In some embodiments, Friedreich's ataxia is associated with a disease-associated repeat of a GAA trinucleotide in the first intron of the FXN gene. In some embodiments, the disease-associated-repeat is expanded to more than sixty repeat units.

**[000264]** 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 Friedreich's ataxia. In some embodiments, a subject having Friedreich's ataxia has a number of disease-associated-repeat units of GAA in the range of ~60 to ~1300. In some embodiments, a subject having Friedreich's ataxia has a number of disease-associated-repeat units of GAA in the range of 90 to 1,000 or more repeat units. In some embodiments, a subject having Friedreich's ataxia has one or more of the following: degeneration of heart muscle, hypertrophic cardiomyopathy, myocardial fibrosis, heart failure, degeneration of nerve fibers in the spinal cord and degeneration of nerve fibers in the peripheral nervous system.

**[000265]** 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 a muscle-targeting agent covalently 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.

**[000266]** 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

- 87 -

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.

**[000267]** In some embodiments, a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently 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.

**[000268]** In some embodiments, a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently 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.

**[000269]** 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.

**[000270]** 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

- 88 -

**[000271]** 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 Friedreich's ataxia. In some embodiments, symptoms associated with Friedreich's ataxia may include degeneration of heart muscle, hypertrophic cardiomyopathy, myocardial fibrosis, heart failure, degeneration of nerve fibers in the spinal cord and degeneration of nerve fibers in the peripheral nervous system.

**[000272]** In some embodiments, a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently 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.

**[000273]** In some embodiments, a single dose or administration of a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently to a molecular payload described herein to a subject is sufficient to inhibit activity or expression of a target gene for at least 1-5, 1-10, 5-15, 10-20, 15-30, 20-40, 25-50, or more days. In some embodiments, a single dose or administration of a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently to a molecular payload described herein to a subject is sufficient to inhibit activity or expression of a target gene for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 weeks. In some embodiments, a single dose or administration of a pharmaceutical composition that comprises a complex comprising a muscle-targeting agent covalently 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.

**[000274]** In some embodiments, a pharmaceutical composition may comprises more than one complex comprising a muscle-targeting agent covalently 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 Friedreich's ataxia. In some embodiments, the other therapeutic agents may enhance or supplement the effectiveness of the complexes described herein. In some embodiments, the other therapeutic agents may function to treat a different symptom or disease than the complexes described herein.

## EXAMPLES

**Example 1: Targeting HPRT with transfected antisense oligonucleotides**

**[000275]** A siRNA that targets hypoxanthine phosphoribosyltransferase (HPRT) was tested *in vitro* for its ability to reduce expression levels of HPRT in an immortalized cell line. Briefly, Hepa 1-6 cells were transfected with either a control siRNA (siCTRL; 100 nM) or the siRNA that targets HPRT (siHPRT; 100 nM), formulated with lipofectamine 2000. HPRT expression levels were evaluated 48 hours following transfection. A control experiment was also performed in which vehicle (phosphate-buffered saline) was delivered to Hepa 1-6 cells in culture and the cells were maintained for 48 hours. As shown in FIG. 1, it was found that the HPRT siRNA reduced HPRT expression levels by ~90% compared with controls.

Table 2. Sequences of siHPRT and siCTRL

	Sequence
siHPRT sense strand	5'-UcCuAuGaCuGuAgAuUuUaU-(CH <sub>2</sub> ) <sub>6</sub> NH <sub>2</sub> -3'
siHPRT antisense strand	5'-paUaAaAuCuAcAgUcAuAgGasAsu-3'
siCTRL sense strand	5'-UgUaAuAaCcAuAuCuAcCuU-(CH <sub>2</sub> ) <sub>6</sub> NH <sub>2</sub> -3'
siCTRL antisense strand	5'-aAgGuAgAuAuGgUuAuUaCasAsa-3'

\*Lower case - 2'Ome ribose; Capital letter - 2'Fluoro ribose; p – phosphate linkage; s – phosphorothioate linkage

**Example 2: Targeting HPRT with a muscle-targeting complex**

**[000276]** A muscle-targeting complex was generated comprising the HPRT siRNA used in Example 1 (siHPRT) covalently linked, via a non-cleavable N-gamma-maleimidobutyl-oxysuccinimide ester (GMBS) linker, to DTX-A-002, an anti-transferrin receptor antibody.

**[000277]** Briefly, the GMBS linker was dissolved in dry DMSO and coupled to the 3' end of the sense strand of siHPRT through amide bond formation under aqueous conditions.

Completion of the reaction was verified by Kaiser test. Excess linker and organic solvents were removed by gel permeation chromatography. The purified, maleimide functionalized sense strand of siHPRT was then coupled to DTX-A-002 antibody using a Michael addition reaction.

**[000278]** The product of the antibody coupling reaction was then subjected to hydrophobic interaction chromatography (HIC-HPLC). antiTfR-siHPRT complexes comprising one or two siHPRT molecules covalently attached to DTX-A-002 antibody were purified. Densitometry confirmed that the purified sample of complexes had an average siHPRT to antibody ratio of 1.46. SDS-PAGE analysis demonstrated that >90% of the purified sample of complexes comprised DTX-A-002 linked to either one or two siHPRT molecules.

- 90 -

**[000279]** Using the same methods as described above, a control IgG2a-siHPRT complex was generated comprising the HPRT siRNA used in Example 1 (siHPRT) covalently linked via the GMBS linker to an IgG2a (Fab) antibody (DTX-A-003). Densitometry confirmed that DTX-C-001 had an average siHPRT to antibody ratio of 1.46 and SDS-PAGE demonstrated that >90% of the purified sample of control complexes comprised DTX-A-003 linked to either one or two siHPRT molecules.

**[000280]** The antiTfR-siHPRT complex was then tested for cellular internalization and inhibition of HPRT *in cellulo*. Hepa 1-6 cells, which have relatively high expression levels of transferrin receptor, were incubated in the presence of vehicle (phosphate-buffered saline), IgG2a-siHPRT (100 nM), antiTfR-siCTRL (100 nM), or antiTfR-siHPRT (100 nM), for 72 hours. After the 72 hour incubation, the cells were isolated and assayed for expression levels of HPRT (FIG. 2). Cells treated with the antiTfR-siHPRT demonstrated a reduction in HPRT expression by ~50% relative to the cells treated with the vehicle control. Meanwhile, cells treated with either of the IgG2a-siHPRT or antiTfR-siCTRL had HPRT expression levels comparable to the vehicle control (no reduction in HPRT expression). These data indicate that the anti-transferrin receptor antibody of the antiTfR-siHPRT enabled cellular internalization of the complex, thereby allowing the siHPRT to inhibit expression of HPRT.

### **Example 3: Targeting HPRT in mouse muscle tissues with a muscle-targeting complex**

**[000281]** The muscle-targeting complex described in Example 2, antiTfR-siHPRT, was tested for inhibition of HPRT in mouse tissues. C57BL/6 wild-type mice were intravenously injected with a single dose of a vehicle control (phosphate-buffered saline); siHPRT (2 mg/kg of RNA); IgG2a-siHPRT (2 mg/kg of RNA, corresponding to 9 mg/kg antibody complex); or antiTfR-siHPRT (2 mg/kg of RNA, corresponding to 9 mg/kg antibody complex). Each experimental condition was replicated in four individual C57BL/6 wild-type mice. Following a three-day period after injection, the mice were euthanized and segmented into isolated tissue types. Individual tissue samples were subsequently assayed for expression levels of HPRT (FIGs. 3A-3B and 4A-4E).

**[000282]** Mice treated with the antiTfR-siHPRT complex demonstrated a reduction in HPRT expression in gastrocnemius (31% reduction;  $p < 0.05$ ) and heart (30% reduction;  $p < 0.05$ ), relative to the mice treated with the siHPRT control (FIGs. 3A-3B). Meanwhile, mice treated

- 91 -

with the IgG2a-siHPRT complex had HPRT expression levels comparable to the siHPRT control (little or no reduction in HPRT expression) for all assayed muscle tissue types.

**[000283]** Mice treated with the antiTfR-siHPRT complex demonstrated no change in HPRT expression in non-muscle tissues such as brain, liver, lung, kidney, and spleen tissues (FIGs. 4A-4E).

**[000284]** These data indicate that the anti-transferrin receptor antibody of the antiTfR-siHPRT complex enabled cellular internalization of the complex into muscle-specific tissues in an *in vivo* mouse model, thereby allowing the siHPRT to inhibit expression of HPRT. These data further demonstrate that the antiTfR-oligonucleotide complexes of the current disclosure are capable of specifically targeting muscle tissues.

#### **Example 4: Muscle-targeting complex targeting FXN alleles**

**[000285]** A muscle-targeting complex is generated comprising an oligonucleotide (e.g., an antisense oligonucleotide that inhibits R-loop formation in a FXN allele) (a FXN ASO) covalently linked, via a cathepsin cleavable linker, to DTX-A-002 (RI7 217 (Fab)), an anti-transferrin receptor antibody.

**[000286]** Briefly, a maleimidocaproyl-L-valine-L-citrulline-p-aminobenzyl alcohol p-nitrophenyl carbonate (MC-Val-Cit-PABC-PNP) linker molecule is coupled to NH<sub>2</sub>-C<sub>6</sub>-FXN ASO using an amide coupling reaction. Excess linker and organic solvents are removed by gel permeation chromatography. The purified Val-Cit-linker-FXN ASO is then coupled to a thiol-reactive anti-transferrin receptor antibody (DTX-A-002).

**[000287]** The product of the antibody coupling reaction is then subjected to hydrophobic interaction chromatography (HIC-HPLC) to purify the muscle-targeting complex. Densitometry and SDS-PAGE analysis of the purified complex allow for determination of the average ratio of ASO-to-antibody and total purity, respectively.

**[000288]** Using the same methods as described above, a control complex is generated comprising FXN ASO covalently linked via a Val-Cit linker to an IgG2a (Fab) antibody.

**[000289]** The purified muscle-targeting complex comprising DTX-A-002 covalently linked to FXN ASO is then tested for cellular internalization and inhibition of R-loop formation in a FXN allele. Disease-relevant muscle cells that have relatively high expression levels of transferrin receptor, are incubated in the presence of vehicle control (saline), muscle-targeting

- 92 -

complex (100 nM), or control complex (100 nM) for 72 hours. After the 72 hour incubation, the cells are isolated and assayed for R-loop formation in a FXN allele.

**[000290]** Similar complexes are produced and evaluated using other oligonucleotides useful for targeting FXN disclosed herein.

#### EQUIVALENTS AND TERMINOLOGY

**[000291]** 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.

**[000292]** 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.

**[000293]** 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 one or more modified nucleotides and/or one or more modified internucleotide linkages and/or one or more other modification compared with the specified sequence while retaining essentially same or similar complementary properties as the specified sequence.

**[000294]** 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

- 93 -

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.

**[000295]** 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.

**[000296]** 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.

- 94 -

## CLAIMS

What is claimed is:

1. A complex comprising a muscle-targeting agent covalently linked to a molecular payload configured for increasing FXN expression, wherein the muscle-targeting agent specifically binds to an internalizing cell surface receptor on muscle cells.
2. The complex of claim 1, wherein the muscle-targeting agent is a muscle-targeting antibody.
3. The complex of claim 2, wherein the muscle-targeting antibody specifically binds to an extracellular epitope of a transferrin receptor.
4. The complex of claim 3, wherein the extracellular epitope of the transferrin receptor comprises an epitope of the apical domain of the transferrin receptor.
5. The complex of claim 3 or 4, wherein the muscle-targeting antibody specifically binds to an epitope of a sequence in the range of C89 to F760 of SEQ ID NO: 1-3.
6. The complex of any one of claims 3 to 5, wherein the equilibrium dissociation constant ( $K_d$ ) of binding of the muscle-targeting antibody to the transferrin receptor is in a range from  $10^{-11}$  M to  $10^{-6}$  M.
7. The complex of any one of claims 3 to 6, wherein the muscle-targeting antibody competes for specific binding to an epitope of a transferrin receptor with an antibody listed in Table 1.
8. The complex of claim 7, wherein the muscle-targeting antibody competes for specific binding to an epitope of a transferrin receptor with a  $K_d$  of less than or equal to  $10^{-6}$  M.
9. The complex of claim 8, wherein the  $K_d$  is in a range of  $10^{-11}$  M to  $10^{-6}$  M.
10. The complex of any one of claims 3 to 9, wherein the muscle-targeting antibody does not specifically bind to the transferrin binding site of the transferrin receptor and/or wherein the muscle-targeting antibody does not inhibit binding of transferrin to the transferrin receptor.

- 95 -

11. The complex of any one of claims 3 to 10, wherein the muscle-targeting antibody is cross-reactive with extracellular epitopes of two or more of a human, non-human primate and rodent transferrin receptor.

12. The complex of any one of claims 3 to 11, wherein the complex is configured to promote transferrin receptor mediated internalization of the molecular payload into a muscle cell.

13. The complex of any one of claims 2 to 12, wherein the muscle-targeting antibody is a chimeric antibody, optionally wherein the chimeric antibody is a humanized monoclonal antibody.

14. The complex of any one of claims 2 to 13, wherein the muscle-targeting antibody is in the form of a ScFv, Fab fragment, Fab' fragment, F(ab')<sub>2</sub> fragment, or Fv fragment.

15. The complex of any one of claims 1 to 14, wherein the molecular payload is an oligonucleotide, optionally wherein the oligonucleotide comprises DNA or RNA.

16. The complex of claim 15, wherein the oligonucleotide comprises a region of complementarity to an FXN allele.

17. The complex of any one of claims 1 to 14, wherein the molecular payload is a polypeptide.

18. The complex of claim 17, wherein the polypeptide comprises frataxin, a fragment of frataxin, or a variant of frataxin.

19. The complex of either of claims 15 or 16, wherein the oligonucleotide comprises an antisense strand having a region of complementarity to a GAA trinucleotide sequence.

20. The complex of either of claims 15 or 16, wherein the oligonucleotide inhibits R-loop formation in a FXN allele.

21. The complex of any one of claims 15, 16, 19, or 20, wherein the oligonucleotide comprises at least one modified internucleotide linkage.

22. The complex of claim 21, wherein the at least one modified internucleotide linkage is a phosphorothioate linkage.

- 96 -

23. The complex of claim 22, wherein the oligonucleotide comprises phosphorothioate linkages in the Rp stereochemical conformation and/or in the Sp stereochemical conformation.

24. The complex of claim 23, wherein the oligonucleotide comprises phosphorothioate linkages that are all in the Rp stereochemical conformation or that are all in the Sp stereochemical conformation.

25. The complex of any one of claims 15, 16, or 19 to 24, wherein the oligonucleotide comprises one or more modified nucleotides.

26. The complex of claim 25, wherein the one or more modified nucleotides are 2'-modified nucleotides.

27. The complex of any one of claims 15, 16, 19, or 20, wherein the oligonucleotide is a mixmer oligonucleotide.

28. The complex of claim 27, wherein the mixmer oligonucleotide comprises two or more different 2' modified nucleotides.

29. The complex of claim 27, wherein each 2' modified nucleotide is selected from the group consisting of: 2'-O-methyl, 2'-fluoro (2'-F), 2'-O-methoxyethyl (2'-MOE), and 2', 4'-bridged nucleotides.

30. The complex of claim 27, wherein the one or more modified nucleotides are bridged nucleotides.

31. The complex of claim 27, wherein at least one 2' modified nucleotide is a 2',4'-bridged nucleotide selected from: 2',4'-constrained 2'-O-ethyl (cEt) and locked nucleic acid (LNA) nucleotides.

32. The complex of claim 15 or 16, wherein the oligonucleotide comprises a guide sequence for a genome editing nuclease.

33. The complex of any one of claims 15, 16, 19, or 20, wherein the oligonucleotide is phosphorodiamidite morpholino oligomer.

34. The complex of claim 15, wherein the oligonucleotide is an mRNA encoding frataxin, a fragment of frataxin, or a variant of frataxin.

- 97 -

35. The complex of claim 34, wherein the mRNA comprises a 5' cap structure and/or a 3' polyA tail.

36. The complex of any one of claims 1 to 35, wherein the muscle-targeting agent is covalently linked to the molecular payload via a cleavable linker.

37. The complex of claim 36, wherein the cleavable linker is selected from: a protease-sensitive linker, pH-sensitive linker, and glutathione-sensitive linker.

38. The complex of claim 37, wherein the cleavable linker is a protease-sensitive linker.

39. The complex of claim 38, wherein the protease-sensitive linker comprises a sequence cleavable by a lysosomal protease and/or an endosomal protease.

40. The complex of claim 38, wherein the protease-sensitive linker comprises a valine-citrulline dipeptide sequence.

41. The complex of claim 37, wherein the linker is pH-sensitive linker that is cleaved at a pH in a range of 4 to 6.

42. The complex of any one of claims 1 to 35, wherein the muscle-targeting agent is covalently linked to the molecular payload via a non-cleavable linker.

43. The complex of claim 42, wherein the non-cleavable linker is an alkane linker.

44. The complex of any of claims 2 to 43, wherein the muscle-targeting antibody comprises a non-natural amino acid to which the oligonucleotide is covalently linked.

45. The complex of any of claims 2 to 43, wherein the muscle-targeting antibody is covalently linked to the oligonucleotide via conjugation to a lysine residue or a cysteine residue of the antibody.

46. The complex of claim 45, wherein the oligonucleotide is conjugated to the cysteine of the antibody via a maleimide-containing linker, optionally wherein the maleimide-containing linker comprises a maleimidocaproyl or maleimidomethyl cyclohexane-1-carboxylate group.

- 98 -

47. The complex of claims 2 to 46, wherein the muscle-targeting antibody is a glycosylated antibody that comprises at least one sugar moiety to which the oligonucleotide is covalently linked.

48. The complex of claim 47, wherein the sugar moiety is a branched mannose.

49. The complex of claim 47 or 48, wherein the muscle-targeting antibody is a glycosylated antibody that comprises one to four sugar moieties each of which is covalently linked to a separate oligonucleotide.

50. The complex of claim 47, wherein the muscle-targeting antibody is a fully-glycosylated antibody.

51. The complex of claim 47, wherein the muscle-targeting antibody is a partially-glycosylated antibody.

52. The complex of claim 51, wherein the partially-glycosylated antibody is produced via chemical or enzymatic means.

53. The complex of claim 52, wherein the partially-glycosylated antibody is produced in a cell that is deficient for an enzyme in the N- or O- glycosylation pathway.

54. A method of delivering a molecular payload to a cell expressing transferrin receptor, the method comprising contacting the cell with the complex of any one of claims 1 to 53.

55. A method of increasing expression of FXN in a cell, the method comprising contacting the cell with the complex of any one of claims 1 to 53 in an amount effective for promoting internalization of the molecular payload to the cell.

56. The method of claim 55, wherein the cell is *in vitro*.

57. The method of claim 55, wherein the cell is in a subject.

58. The method of claim 57, wherein the subject is a human.

59. A method of treating a subject having a mutant FXN allele that is associated with Friedreich's ataxia, the method comprising administering to the subject an effective amount of the complex of any one of claims 1 to 53.

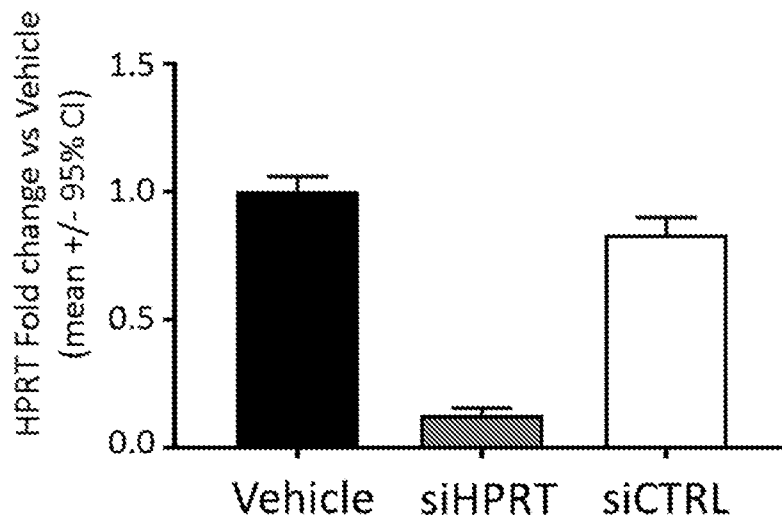


FIG. 1

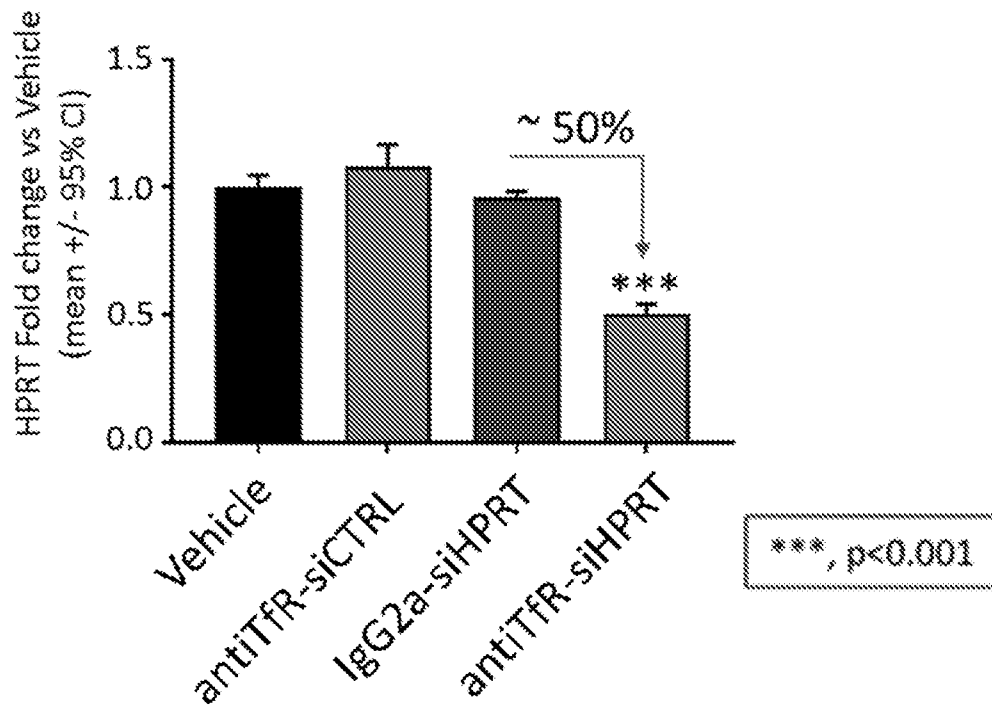


FIG. 2

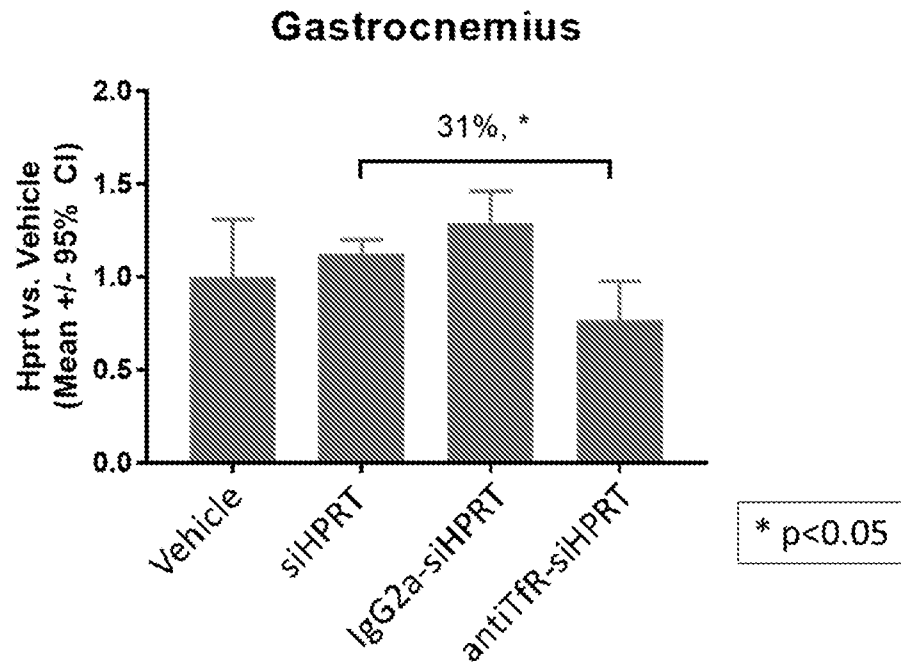


FIG. 3A

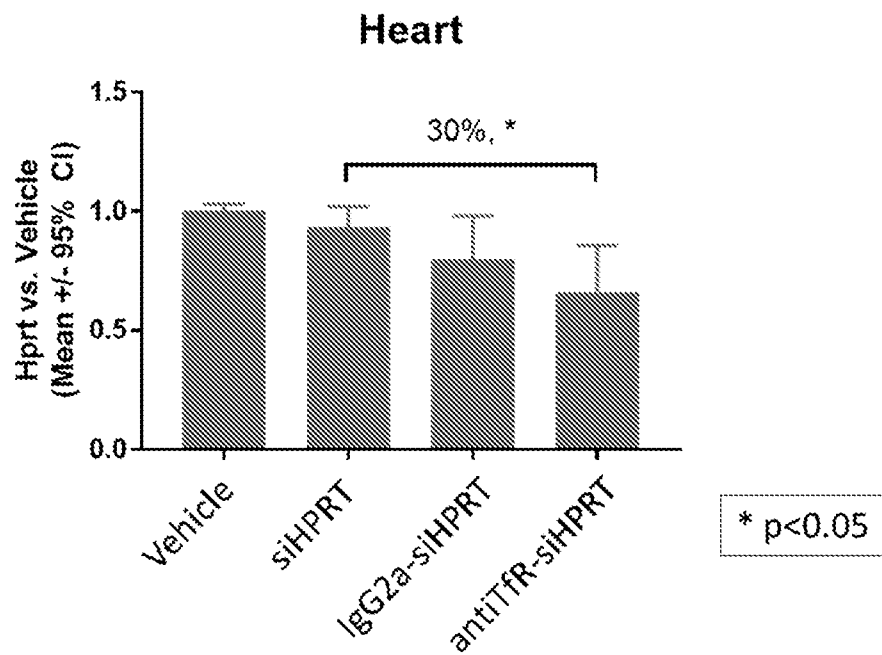


FIG. 3B

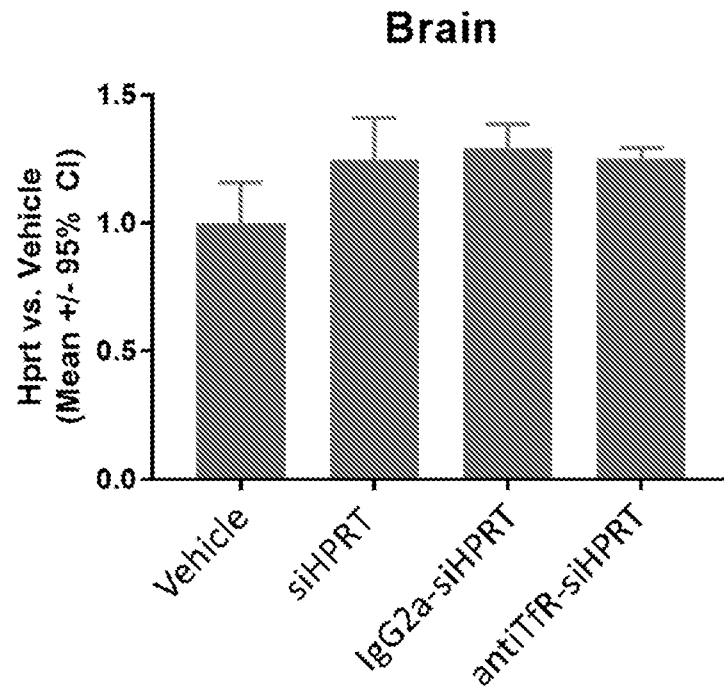


FIG. 4A

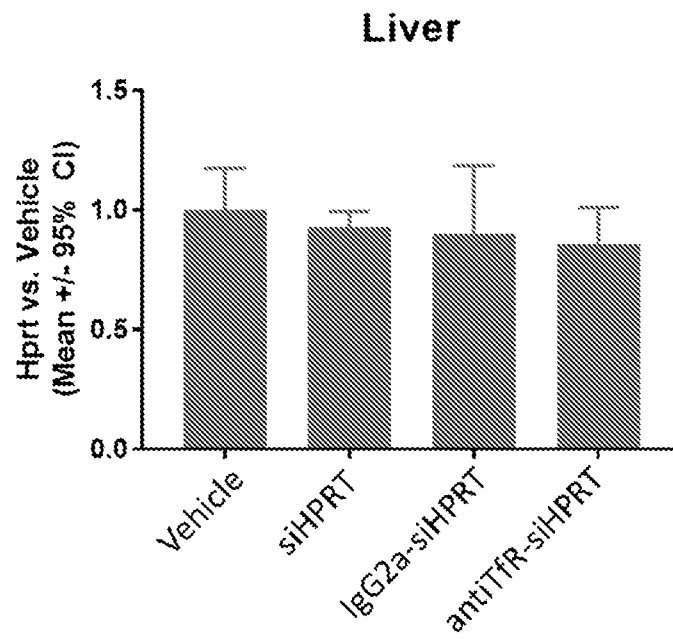


FIG. 4B

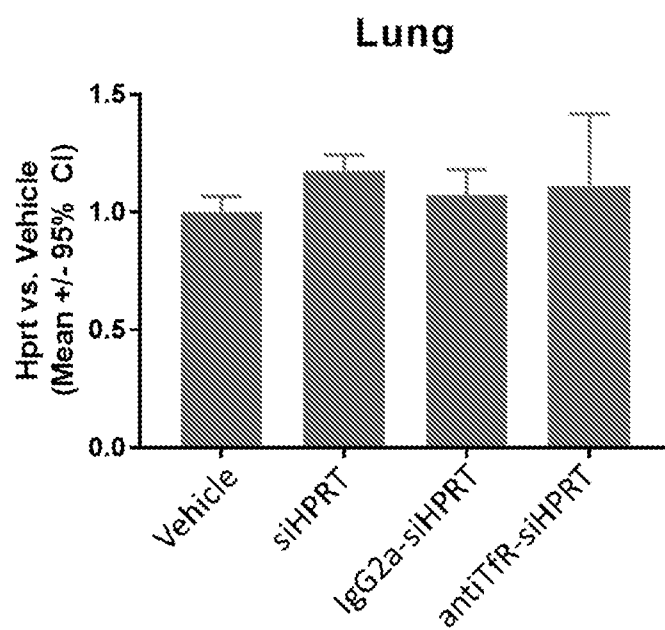


FIG. 4C

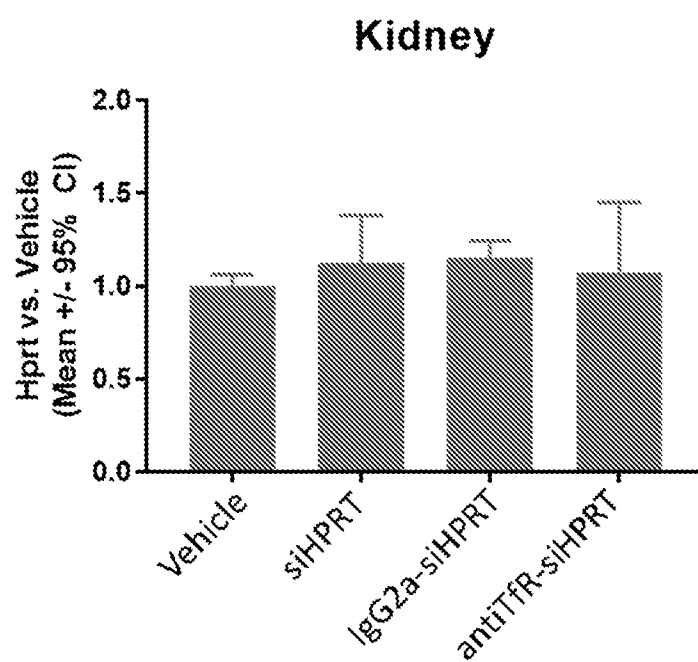
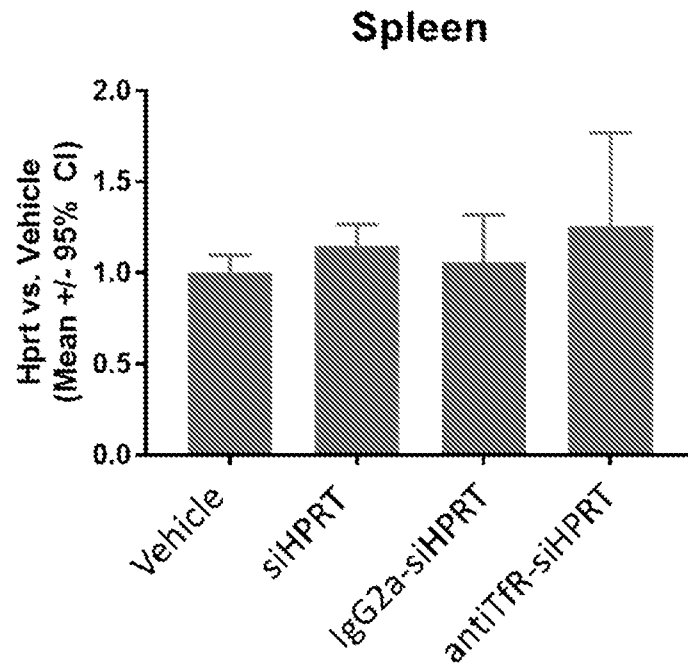


FIG. 4D

**FIG. 4E**

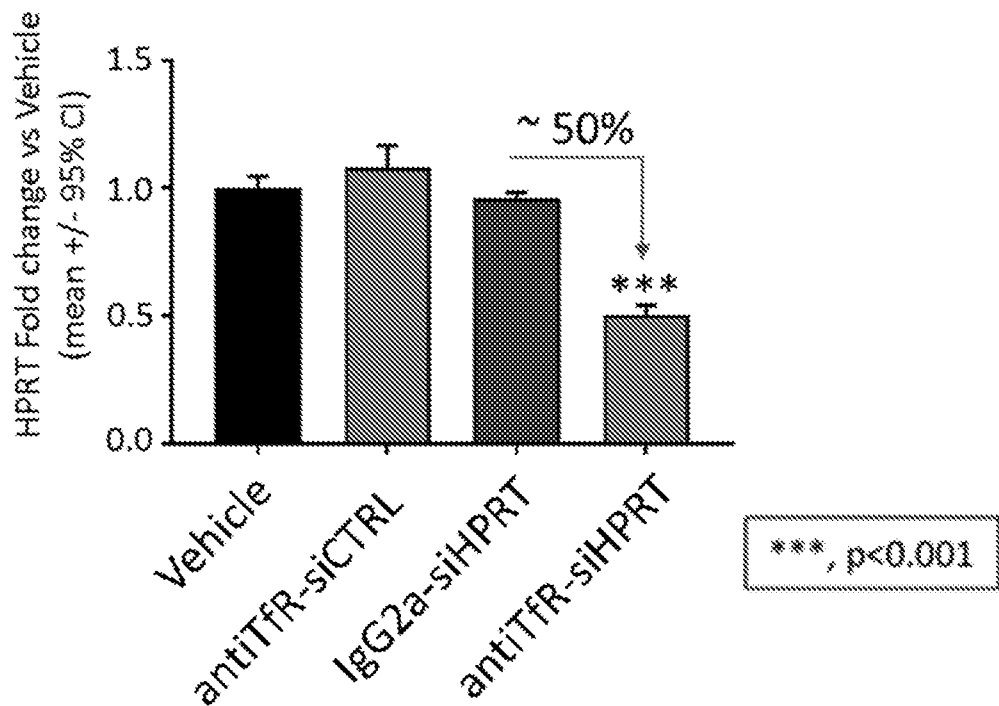


FIG. 2