

ANTI-TRANSFERRIN RECEPTOR ANTIBODIES AND USES THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

[001] The present application claims the benefit of U.S. Provisional Application No. 5 63/545,848, filed October 26, 2023, and U.S. Provisional Application No. 63/549,055, filed February 2, 2024, the entire contents of which are hereby incorporated by reference herein.

TECHNICAL FIELD

[002] This disclosure relates to anti-transferrin receptor antibodies, compositions 10 comprising the same, and methods of use for the delivery of therapeutic cargo to brain tissue. This disclosure also provides related polynucleotides and vectors encoding the anti-transferrin receptor antibodies and cells comprising the same.

BACKGROUND

15 [003] The delivery of drugs to the central nervous system has been a challenge in the treatment of neurological diseases such as Alzheimer's disease and Parkinson's disease. For drugs to reach the brain, they first have to penetrate the blood brain barrier, which is a major challenge due to the selectivity of the blood brain barrier. The blood brain barrier acts as a semipermeable membrane, preventing most molecules from entering the nervous system from 20 the blood and allows only low molecular weight (<400 Da) and lipophilic compounds to pass. Most small molecules and large molecules, such as monoclonal antibodies and antisense oligonucleotides, cannot pass through this barrier. Due to this challenging process of drug penetration across the blood brain barrier, a small fraction of therapeutic agents for neurological diseases make it to clinical trials.

25 [004] There is a need in the art for improved compositions and methods for delivering a therapeutic agent to the central nervous system.

SUMMARY

[005] This disclosure relates to anti-transferrin receptor antibodies and methods of their 30 use for the delivery of cargo to brain tissue and the treatment of neurological disorders.

[006] Provided herein is an antibody that binds to human transferrin receptor, comprising a heavy chain variable region (VH) comprising VH complementarity determining region (CDR)1, VH CDR2, and VH CDR3, and a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3, wherein: the VH CDR1 comprises the amino acid sequence GFTFSSYX₁MN (SEQ ID NO:18) or the amino acid sequence SYX₁MN (SEQ ID NO:26), wherein X₁ is S or A; the VH CDR2 comprises the amino acid sequence SISX₂SSSX₃IYYADSVKG (SEQ ID NO:19), wherein X₂ is S or A, and wherein X₃ is Y or S; and the VH CDR3 comprises the amino acid sequence KX₄X₅X₆GDFDY (SEQ ID NO:20), wherein X₄ is Y or S, wherein X₅ is R or S, and wherein X₆ is A or Y; the VL CDR1 comprises the amino acid sequence RASQSVSSX₇X₈LA (SEQ ID NO:21), wherein X₇ is S or N, and wherein X₈ is Y or N; the VL CDR2 comprises the amino acid sequence GASX₉RAT (SEQ ID NO:22), wherein X₉ is N or S; and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8). In some instances, at least one of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1, and any of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3 that are not selected from the mutant CDRs depicted in Table 1 are selected from the parental CDRs depicted in Table 1. In some instances, one of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3 is selected from the mutant CDRs depicted in Table 1 and five of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1. In some instances, two of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and four of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1. In some instances, three of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and three of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1. In some instances, four of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and two of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1. In some instances, five of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and one of VH CDR1, VH CDR2,

VH CDR3, VL CDR1, VL CDR2, or VL CDR3 is selected from the parental CDRs depicted in Table 1.

[007] In some instances, the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence
 5 SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8); or

10 the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
 15 the amino acid sequence QQQSSSPPT (SEQ ID NO:8).

[008] In some instances, the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence
 20 RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence GFTFSSYAMN (SEQ ID NO:9); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the
 25 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
 30 VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1

comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
5 VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S
10 conservative substitution;

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2
15 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISSSSSSYIYYADSVKG (SEQ ID NO:11); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
20 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISSSSSSYIYYADSVKG (SEQ ID NO:4); the
25 VH CDR3 comprises the amino acid sequence KSRAGDFDY (SEQ ID NO:12); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
30 VH CDR2 comprises the amino acid sequence SISSSSSSYIYYADSVKG (SEQ ID NO:4); the
VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1

comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
 5 VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRYGDFDY (SEQ ID NO:14); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

10 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:25); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
 15 the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
 20 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
 25 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the
 30 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNLA (SEQ ID NO:16); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the
 5 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASSRAT (SEQ ID NO:17); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH
 10 CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S
 15 conservative substitution;

the VH CDR1 comprises the amino acid sequence SYAMN (SEQ ID NO:24); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
 20 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
 25 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH
 30 CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH
5 CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

10 the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:11); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
15 the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KSRAGDFDY (SEQ ID NO:12); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
20 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1
25 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH
30 CDR3 comprises the amino acid sequence KYRYGDFDY (SEQ ID NO:14); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSIYYADSVKG (SEQ ID NO:25); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNLA (SEQ ID NO:16); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8); or

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASSRAT (SEQ ID NO:17); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8).

[009] In some instances, (i) the VH is at least 80%, 85%, 90%, 95%, 99%, or 100% identical to any one of SEQ ID NOs:100-108; and (ii) the VL is at least 80%, 85%, 90%, 95%,
5 99%, or 100% identical to any one of SEQ ID NOs:200-204. In some instances, the VH comprises the amino acid sequence of any one of SEQ ID NOs:100-108 and the VL comprises the amino acid sequence of any one of SEQ ID NOs:200-204.

[0010] In some instances, the antibody is (a) monovalent and has a monovalent affinity (K_D) for hTfR1 of > 10 nM or is bivalent and has a monovalent affinity (K_D) for hTfR1 of >100
10 nM and/or (b) has an off rate (k_d) of $\geq 0.01/s$.

[0011] In some instances, the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:200.

[0012] In some instances, the VH comprises the amino acid sequence of SEQ ID NO:101 and the VL comprises the amino acid sequence of SEQ ID NO:200; the VH comprises the amino
15 acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO: 200; the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO:201; the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO:203; the VH
20 comprises the amino acid sequence of SEQ ID NO:103 and the VL comprises the amino acid sequence of SEQ ID NO:200; the VH comprises the amino acid sequence of SEQ ID NO:104 and the VL comprises the amino acid sequence of SEQ ID NO:200; the VH comprises the amino acid sequence of SEQ ID NO:105 and the VL comprises the amino acid sequence of SEQ ID NO:200; the VH comprises the amino acid sequence of SEQ ID NO:106 and the VL comprises the amino acid sequence of SEQ ID NO:200; the VH comprises the amino acid sequence of SEQ
25 ID NO:107 and the VL comprises the amino acid sequence of SEQ ID NO:200; the VH comprises the amino acid sequence of SEQ ID NO:108 and the VL comprises the amino acid sequence of SEQ ID NO:200; the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID
30 NO:202; the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises

the amino acid sequence of SEQ ID NO:203; or the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:204.

[0013] In some instances, the antibody is a multispecific antibody, bispecific antibody, single chain antibody, an Fab fragment, an F(ab')₂ fragment, an Fab' fragment, an Fsc fragment, an Fv fragment, an scFv, an sc(Fv)₂, or a diabody.

[0014] In some instances, the antibody comprises a constant heavy chain (CH) domain and a constant light chain (CL) domain.

[0015] In some instances, the antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises or consists of the amino acid sequences set forth in SEQ ID NO:400 and the light chain comprises or consists of the amino acid sequence set forth in SEQ ID NO:401.

[0016] In some instances, the antibody comprises a Fab fragment. In some instances, the antibody comprises a Fab fragment, wherein the Fab fragment comprises the amino acid sequences set forth in SEQ ID NOs:401 and 402.

[0017] In some instances, the antibody comprises a Fab-Fc. In some instances, the antibody comprises a Fab-Fc, wherein the Fab-Fc comprises the amino acid sequences set forth in SEQ ID NOs:401, 404, and 406. In some instances, the antibody comprises a Fab-Fc, wherein the Fab-Fc comprises the amino acid sequences set forth in SEQ ID NOs: 400 and 401.

[0018] In some instances, the antibody comprises an Fc-Fab. In some instances, the antibody comprises an Fc-Fab, wherein the Fc-Fab comprises the amino acid sequences set forth in SEQ ID NOs:401, 405, and 406. In some instances, the antibody comprises an Fc-Fab, wherein the Fc-Fab comprises the amino acid sequences set forth in SEQ ID NOs: 401 and 403.

[0019] In some instances, the antibody comprises: (a) a heavy chain comprising the amino acid sequence set forth in 400, and a light chain comprising the amino acid sequence set forth in 401; (b) the amino acid sequences set forth in SEQ ID NOs: 401 and 402; (c) the amino acid sequences set forth in SEQ ID NOs: 401, 404, and 406; (d) the amino acid sequences set forth in SEQ ID NOs:400 and 401; (e) the amino acid sequences set forth in SEQ ID NOs: 401, 405, and 406; or (f) the amino acid sequences set forth in SEQ ID NOs: 401 and 403.

[0020] Also provided herein is a nucleic acid or nucleic acids encoding any one of the foregoing antibodies.

[0021] Also provided herein is an expression vector or expression vectors comprising the foregoing nucleic acid or nucleic acids operably linked to a promoter.

5 [0022] Also provided herein is an isolated cell comprising the foregoing nucleic acid or nucleic acids or the foregoing expression vector or expression vectors.

[0023] Also provided herein is an isolated cell comprising a first expression vector comprising a first nucleic acid encoding a first polypeptide comprising the VH of any one of the foregoing antibodies operably linked to a promoter, and a second expression vector comprising a
10 second nucleic acid encoding a second polypeptide comprising the VL of any one of the foregoing antibodies operably linked to a promoter.

[0024] Also provided herein is a method of making any one of the foregoing antibodies, comprising culturing the foregoing cell and isolating the antibody.

[0025] Also provided herein is a pharmaceutical composition comprising of any one of
15 the foregoing antibodies and a pharmaceutically acceptable carrier.

[0026] Also provided herein is a conjugate comprising of any one of the foregoing antibodies and an agent. In some instances, the agent is an antibody, protein, or peptide. In some instances, the agent is an anti-beta amyloid antibody. In some instances, the anti-beta amyloid antibody is aducanumab, bapineuzumab, gantenerumab, solanezumab, donanemab, or
20 lecanemab. In some instances, the agent is an anti-tau antibody, an anti-alpha synuclein antibody, an anti-TDP-43 antibody, an anti-LINGO-1 antibody, an anti-LINGO-2 antibody, an anti-LINGO-3 antibody, an anti-LINGO-4 antibody, an anti-TREM2 antibody, or an anti-C9orf72 dipeptide repeat poly-GA antibody. In some instances, the agent is protein. In some instances, the protein is progranulin. In some instances, the agent is an enzyme. In some
25 instances, the enzyme is glucocerebrosidase. In some instances, the conjugate is a recombinant fusion protein comprising the antibody and the agent. In some instances, the agent is a nucleic acid. In some instances, the nucleic acid is an mRNA, a siRNA, an antisense oligonucleotide, microRNA (miRNA), guide RNA (gRNA), or a phosphoroamidate morpholino oligomer (PMO).

In some instances, the nucleic acid is linked to the antibody via a linker. In some instances, the agent is a nanoparticle, liposome, or viral vector.

[0027] Also provided herein is a method of transporting an agent across the blood brain barrier via transcytosis, the method comprising administering to a human subject any one of the foregoing conjugates.

[0028] Also provided herein is a method of delivering an agent in vivo, the method comprising administering to a human subject any one of the foregoing conjugates. In some instances, the human subject has a neurological disorder and the method delivers the agent to brain tissue. In some instances, the neurological disorder is Alzheimer's disease, Parkinson's disease, frontotemporal dementia, ALS, Huntington's disease, multiple sclerosis, spinal muscular atrophy, muscular dystrophy, spinal cord injury, stroke, an ophthalmological condition, acute or chronic optic neuritis, a psychiatric disorder, Tourette's disease brain injury, a brain tumor, or epilepsy.

[0029] Also provided herein is a method of treating Alzheimer's disease in a human subject in need thereof, comprising administering to the human subject a therapeutically effective amount of any one of the foregoing conjugates.

[0030] Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031] FIG. 1A is a graph depicting the Fab binding response (resonance units, RU) for ANTIBODY X and human transferrin receptor 1 ectodomain (ECD) measured by surface plasmon resonance.

[0032] FIG. 1B is a graph depicting the Fab binding response (resonance units, RU) for ANTIBODY X and cynomolgus monkey transferrin receptor 1 ectodomain measured by surface plasmon resonance.

[0033] FIG. 1C is a graph depicting the fraction bound (normalized mean fluorescent intensity, MFI) of CHO cells expressing human TfR1 by the indicated ANTIBODY X formats, following a 1h incubation on ice, measured by flow cytometry.

[0034] FIG. 2A is a graph depicting the antibody bound (MFI) to hTfR1 expressed on CHO cells for ANTIBODY X alone or in combination with holo human transferrin (holoTf).

[0035] FIG. 2B is a graph depicting displacement of AlexaFluor647 (AF647)-labeled human Tf from hTfR1 CHO cells by ANTIBODY X, transferrin-blocking antibody (Antibody 1), or holo-Tf.

[0036] FIG. 3A is an atomic model of ANTIBODY X Fab bound to the human transferrin receptor in the presence of the endogenous ligand transferrin built into the cryo-EM density at 3.6 Å resolution.

[0037] FIG. 3B is a partial alignment of TfR1 apical and protease-like domain sequences for cynomolgus monkey TfR and human TfR, with residues within 4 Å of ANTIBODY X, as observed in the cryo-EM structure of FIG. 3A, highlighted in gray and the conservative R364K mutation (hu>cyno TfR1) within the ANTIBODY X epitope in bold. Strictly conserved residues are denoted by asterisk, strongly conserved residues by colon, and moderately conserved residues by period. Sources of sequences: Human (*Homo sapiens*, Uniprot P02786.2); Cynomolgus (*Macaca fascicularis*, NCBI Reference Sequence: XP_045243212.1).

[0038] FIG. 4A is a graph depicting the Fab binding response (resonance units, RU; y axis) for ANTIBODY X in Fab-Fc monovalent format and hTfR1 ECD, measured at pH 7.4 and pH 5.5.

[0039] FIG. 4B is a graph depicting Caco2 transcytosis of ANTIBODY X in indicated antibody formats.

[0040] FIG. 5A is a graph depicting serum hIgG concentration (nM) at the indicated times following IV administration of ANTIBODY X (squares) or control hIgG (circles) at 20 mg/kg in hTfR KI mice.

[0041] FIG. 5B is a graph depicting brain hIgG concentration (nM) at the indicated times following IV administration of ANTIBODY X (squares) or control hIgG (circles) at 20 mg/kg in hTfR KI mice.

[0042] FIG. 5C is a hIgG immunohistochemistry image of brain samples from hTfR KI mice three days after being administered ANTIBODY X or control hIgG.

[0043] FIG. 5D is an image of immunohistochemistry staining for hIgG in the cerebral cortex from hTfR KI mice administered ANTIBODY X.

[0044] FIG. 6A is a graph depicting hIgG levels in cynomolgus monkey brain biopsies 48h after treatment with 20mg/kg IV of hIgG or ANTIBODY X.

[0045] FIG. 6B are images of cynomolgus monkey brain biopsies, showing hIgG staining by immunohistochemistry for hippocampus, cerebellum, and cerebral cortex 48h after administration of 20 mg/kg IV of a negative control or ANTIBODY X.

[0046] FIG. 6C is an image of a cynomolgus monkey brain biopsy after administration of ANTIBODY X, showing vesicles within pyramidal neurons of the cerebral cortex.

[0047] FIG. 7 is a series of cartoons depicting control hIgG (left), anti-BACE1 (second from left), anti-BACE1/ANTIBODY X bivalent (third from left), and anti-BACE1/ANTIBODY X monovalent (right).

[0048] FIG. 8A is a graph depicting plasma hIgG concentration (nM) at the indicated timepoints in hTfR KI mice administered 50 mg/kg IV molar IgG equivalent of hIgG (squares), anti-BACE (circles), anti-BACE1/ANTIBODY X bivalent (triangles, pointing down), or anti-BACE1/ANTIBODY X monovalent (triangles, pointing up).

[0049] FIG. 8B is a graph depicting brain hIgG concentration (nM) at the indicated timepoints in hTfR KI mice administered 50 mg/kg IV molar IgG equivalent of hIgG (squares), anti-BACE (circles), anti-BACE1/ANTIBODY X bivalent (triangles, pointing down), or anti-BACE1/ANTIBODY X monovalent (triangles, pointing up).

[0050] FIG. 8C is a graph depicting brain amyloid beta 1-40 (A β 40; % control) at the indicated timepoints in hTfR KI mice administered 50 mg/kg IV molar IgG equivalent of hIgG (squares), anti-BACE (circles), anti-BACE1/ANTIBODY X bivalent (triangles, pointing down), or anti-BACE1/ANTIBODY X monovalent (triangles, pointing up).

[0051] FIG. 9A is a graph depicting total hIgG concentration (nM) in hTfR KI mice 24h after being administered the indicated construct (see key in FIG. 9E) in serum.

[0052] FIG. 9B is a graph depicting total hIgG concentration (nM) in hTfR KI mice 24 h after being administered the indicated construct (see key in FIG. 9E) in brain.

[0053] FIG. 9C is a graph depicting free ASO concentration (nM) in hTfR KI mice 24h after being administered the indicated construct (see key in FIG. 9E) in serum.

[0054] FIG. 9D is a graph depicting free ASO concentration (nM) in hTfR KI mice three days after being administered four weekly doses of 1.5 mg/kg ASO equivalents of the indicated construct (see key in FIG. 9E) in brain.

- [0055] FIG. 9E is a graph depicting Malat1 RNA (percent relative to DPBS vehicle mice), normalized to Actb, in hTfR KI mice three days after being administered four weekly doses of 1.5 mg/kg ASO equivalent of the indicated construct, in brain, spinal cord, tibialis anterior, and liver.
- 5 [0056] FIG. 10 is an atomic model of the ANTIBODY X variable domains built using a cryo-electron microscopy density of the ANTIBODY X Fab/TfR1/Tf ternary complex (shown in FIG. 3A).
- [0057] FIG. 11 are graphs depicting the antibody bound (MFI) to CHO cells expressing human (hu) or cynomolgus monkey (cy) TfR1 by the indicated ANTIBODY X mutants.
- 10 [0058] FIG. 12 are graphs depicting the Fab binding response (resonance units, RU; y axis) for Fab fragments of ANTIBODY X variants to hTfR1 ECD, measured at pH 7.4 and pH 5.5 by surface plasmon resonance.
- [0059] FIG. 13A is a graph depicting transcytosis of bivalent ANTIBODY X and ANTIBODY X mutants or control hIgG.
- 15 [0060] FIG. 13B is a graph depicting bivalent transcytosis of ANTIBODY X mutants plotted against the monovalent affinity for hTfR1.
- [0061] FIG. 14 is a series of graphs depicting hIgG concentration (nM) in serum (top row) or in brain (middle row) and percentage of reticulocytes in whole blood (bottom row) at 1 day (left column) or 7 days (right column) post-administration of the indicated constructs at 20
- 20 mg/kg IgG equivalent in hTfR KI mice.
- [0062] FIG. 15A is an image of a capillary Western blot for hTfR1 in Caco2 cell lysate following treatment with 1uM of the indicated antibodies. Baf: bafilomycin.
- [0063] FIG. 15B is a graph depicting TfR1 levels (% of control) in Caco2 cells treated with 1uM of the indicated constructs for 24h, plotted against the EC₅₀ of hTfR1-CHO cell
- 25 binding for each antibody.
- [0064] FIG. 15C is a graph depicting reticulocyte levels (% of control) in hTfR KI mice 24h after being administered 20 mg/kg IV of the indicated constructs (see key in FIG. 15B) plotted against the TfR1 levels in Caco2 after treatment with 1uM of these antibodies for 24h.
- [0065] FIG. 16A is a graph depicting total HPRT mRNA (%) in HEK293T cells after
- 30 72h treatment with siRNA alone or the indicated conjugates at various concentrations.

[0066] FIG. 16B is a graph depicting transcytosis of monovalent ANTIBODY X or control hIgG with and without siRNA.

[0067] FIG. 16C is a graph depicting HPRT RNA (percent relative to DPBS vehicle mice), normalized to Actb, in brain of hTfR KI mice three days after being administered four weekly doses of siRNA or siRNA conjugates at 4 mg/kg siRNA equivalents.

[0068] FIG. 17A is a graph depicting hIgG concentration (nM) in serum at the indicated doses (3, 10, 30, and 90 mg/kg IV molar IgG equivalent) in hTfR KI mice at 1 day post-administration of hIgG control (white circles), or ANTIBODY X monovalent (black circles).

[0069] FIG. 17B is a graph depicting brain hIgG concentration (nM) at the indicated doses (3, 10, 30, and 90 mg/kg IV molar IgG equivalent) in hTfR KI mice at 1 day post-administration of hIgG control (white circles), or ANTIBODY X monovalent (black circles).

[0070] FIG. 17C is a graph depicting brain hIgG concentration (nM) expressed as fold-over-control at the indicated doses (3, 10, 30, and 90 mg/kg IV molar IgG equivalent) in hTfR KI mice at 1 day post-administration of hIgG control or ANTIBODY X monovalent.

[0071] FIG. 18A is a series of cartoons depicting control hIgG (left) and bispecific antibody (right) comprising ANTIBODY Z (bivalent)/ANTIBODY X (monovalent).

[0072] FIG. 18B is a graph depicting serum hIgG concentration (nM) at the indicated timepoints in hTfR KI mice administered 3 mg/kg IV molar IgG equivalent of hIgG control (squares) or four bispecific antibodies incorporating ANTIBODY X variant Fab fragments (ANTIBODY Z bivalent/ANTIBODY X monovalent (VH-S60A) (triangles, pointing up), ANTIBODY Z bivalent/ANTIBODY X monovalent (VL-S39N) (triangles, pointing down), ANTIBODY Z bivalent/ANTIBODY X monovalent (VH-S60A/VL-S39N) (diamonds) or ANTIBODY Z bivalent/ANTIBODY X monovalent (VL-Y57S) (circles)).

[0073] FIG. 18C is a graph depicting brain hIgG concentration (nM) at the indicated timepoints in hTfR KI mice administered 3 mg/kg IV molar IgG equivalent of hIgG control (squares) or four bispecific antibodies incorporating ANTIBODY X variant Fab fragments (ANTIBODY Z bivalent/ANTIBODY X monovalent (VH-S60A) (triangles, pointing up), ANTIBODY Z bivalent/ANTIBODY X monovalent (VL-S39N) (triangles, pointing down), ANTIBODY Z bivalent/ANTIBODY X monovalent (VH-S60A/VL-S39N) (diamonds) or ANTIBODY Z bivalent/ANTIBODY X monovalent (VL-Y57S) (circles)).

[0074] FIG. 18D is a graph depicting reticulocyte levels (% of control) at the indicated timepoints in hTfR KI mice administered 3 mg/kg IV molar IgG equivalent of hIgG control (squares) or four bispecific antibodies incorporating ANTIBODY X variant Fab fragments (ANTIBODY Z bivalent/ANTIBODY X monovalent (VH-S60A) (triangles, pointing up),
5 ANTIBODY Z bivalent/ANTIBODY X monovalent (VL-S39N) (triangles, pointing down), ANTIBODY Z bivalent/ANTIBODY X monovalent (VH-S60A/VL-S39N) (diamonds) or ANTIBODY Z bivalent/ANTIBODY X monovalent (VL-Y57S) (circles)).

DETAILED DESCRIPTION

10 [0075] The present disclosure provides antibodies that specifically bind transferrin receptor 1 (TfR1). Related polypeptides, polynucleotides, vectors, cells, compositions and conjugates comprising the antibodies, methods of making the antibodies, and methods of delivering the compositions and conjugates are also provided. The disclosure also provides methods of using the anti-TfR antibodies.

DEFINITIONS

15 [0076] Unless otherwise defined herein, technical and scientific terms used in the present description have the meanings that are commonly understood by those of ordinary skill in the art. For purposes of interpreting this specification, the following description of terms will apply and
20 whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any description of a term set forth conflicts with any document incorporated herein by reference, the description of the term set forth below shall control.

[0077] The term “antibody” as used herein refers to an immunoglobulin molecule, or a molecule comprising a fragment of an immunoglobulin molecule, that recognizes and binds a
25 target through at least one antigen-binding site. “Antibody” is used herein in the broadest sense and encompasses various antibody structures, including “antibody fragments” and “antigen-binding fragments.” Thus, the term “antibody” includes, but is not limited to, recombinant antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, human
30 antibodies, bispecific antibodies, multispecific antibodies, diabodies, tribodies, tetrabodies, single chain Fv (scFv) antibodies, and antibody fragments as long as they exhibit the desired antigen-binding activity.

[0078] The term “intact antibody” or “full-length antibody” refers to an antibody having a structure substantially similar to a native antibody structure. This includes, for example, an antibody comprising two light chains each comprising a variable region and a light chain constant region (CL) and two heavy chains each comprising a variable region and at least heavy chain constant regions CH1, CH2, and CH3 and a hinge region between CH1 and CH2 regions.

[0079] The term “antigen-binding fragment”, as used herein refers to a molecule other than an intact antibody that comprises a portion of an antibody and an antigen-binding site. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv, single chain antibody molecules (e.g., scFv, sc(Fv)₂), disulfide-linked scFv (dsScFv), diabodies, tribodies, tetrabodies, minibodies, dual variable domain antibodies (DVD), single variable domain antibodies (e.g., camelid antibodies), and multispecific antibodies formed from antibody fragments.

[0080] The term “monoclonal antibody” as used herein refers to a substantially homogenous antibody population involved in the highly specific recognition and binding of a single antigenic determinant or epitope. The term “monoclonal antibody” encompasses intact and full-length monoclonal antibodies as well as antibody fragments (e.g., Fab, Fab', F(ab')₂, Fv), single chain antibodies (e.g., scFv), fusion proteins comprising an antibody fragment, and any other modified immunoglobulin molecule comprising at least one antigen-binding site. Furthermore, “monoclonal antibody” refers to such antibodies made by any number of techniques, including but not limited to, hybridoma production, phage library display, recombinant expression, and transgenic animals.

[0081] The term “chimeric antibody” refers to an antibody in which a portion of the heavy and/or light chain is derived from a first source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0082] The term “humanized antibody” as used herein refers to an antibody that comprises a human heavy chain variable region and a light chain variable region wherein the native CDR amino acid residues are replaced by residues from corresponding CDRs from a nonhuman antibody (e.g., mouse, rat, rabbit, or nonhuman primate), wherein the nonhuman antibody has the desired specificity, affinity, and/or activity. In some embodiments, one or more framework region amino acid residues of the human heavy chain or light chain variable regions are replaced by corresponding residues from nonhuman antibody. Furthermore, humanized

antibodies can comprise amino acid residues that are not found in the human antibody or in the nonhuman antibody. In some embodiments, these modifications are made to further refine and/or optimize antibody characteristics. In some embodiments, the humanized antibody comprises at least a portion of an immunoglobulin constant region (e.g., CH1, hinge, CH2, CH3, Fc), typically that of a human immunoglobulin.

[0083] The term “human antibody” as used herein refers to an antibody that possesses an amino acid sequence that corresponds to an antibody produced by a human and/or an antibody that has been made using any of the techniques that are known to those of skill in the art for making human antibodies. These techniques include, but not limited to, phage display libraries, yeast display libraries, transgenic animals, recombinant protein production, and B-cell hybridoma technology.

[0084] The terms “epitope” and “antigenic determinant” are used interchangeably herein and refer to that portion of an antigen or target capable of being recognized and bound by a particular antibody. When the antigen or target is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of the protein. Epitopes formed from contiguous amino acids (also referred to as linear epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding (also referred to as conformational epitopes) are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5, 6, 7, or 8-10 amino acids in a unique spatial conformation. Epitopes can be predicted using any one of a large number of software bioinformatic tools available on the internet. X-ray crystallography or electron microscopy (e.g., cryo-electron microscopy) may be used to characterize an epitope on a target protein by analyzing the amino acid residue interactions of an antigen/antibody complex.

[0085] The term “specifically binds” or “binds” as used herein refers to an antibody that interacts more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to a particular antigen, epitope, protein, or target molecule than with alternative substances. An antibody that specifically binds an antigen can be identified, for example, by immunoassays, ELISAs, surface plasmon resonance (SPR), or other techniques known to those of skill in the art. In some embodiments, an antibody that specifically binds an antigen (e.g., human TfR1) can bind related antigens (e.g., cyno TfR1). An antibody that specifically binds an antigen can bind the target antigen at a higher affinity than its affinity for a

different antigen. The different antigen can be a related antigen. In some embodiments, an antibody that specifically binds an antigen can bind the target antigen with an affinity that is at least 20 times greater, at least 30 times greater, at least 40 times greater, at least 50 times greater, at least 60 times greater, at least 70 times greater, at least 80 times greater, at least 90 times greater, or at least 100 times greater, than its affinity for a different antigen. In some embodiments, an antibody that specifically binds a particular antigen binds a different antigen at such a low affinity that binding cannot be detected using an assay described herein or otherwise known in the art. In some embodiments, affinity is measured using SPR technology in a Biacore system as described herein or as known to those of skill in the art.

10 **[0086]** The terms “polypeptide” and “peptide” and “protein” are used interchangeably herein and refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid, including but not limited to, unnatural amino acids, as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure may be based upon antibodies, the term “polypeptide” encompasses polypeptides as a single chain and polypeptides of two or more associated chains.

20 **[0087]** The terms “polynucleotide” and “nucleic acid” and “nucleic acid molecule” are used interchangeably herein and refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase.

25 **[0088]** The terms “identical” or percent “identity” in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity may be measured using sequence comparison software or algorithms or by visual inspection. Various

algorithms and software that may be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two nucleic acids or polypeptides of the disclosure are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the sequences that is at least about 10, at least about 20, at least about 20-40, at least about 40-60 nucleotides or amino acid residues, at least about 60-80 nucleotides or amino acid residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 nucleotides or amino acid residues, such as at least about 80-100 nucleotides or amino acid residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, for example, (i) the coding region of a nucleotide sequence or (ii) an amino acid sequence.

[0089] The phrase “conservative amino acid substitution” as used herein refers to a substitution in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been generally defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is considered to be a conservative substitution. Generally, conservative substitutions in the sequences of polypeptides and/or antibodies do not abrogate the binding of the polypeptide or antibody to the target binding site. Methods of identifying nucleotide and amino acid conservative substitutions that do not eliminate binding are well-known in the art.

[0090] The term “vector” as used herein means a construct that is capable of delivering, and usually expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors,

plasmid, cosmid, or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, and DNA or RNA expression vectors encapsulated in liposomes.

[0091] The term “isolated” as used herein refers to a polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition that is in a form not found in nature. An “isolated” antibody is substantially free of material from the cellular source from which it is derived. In some embodiments, isolated polypeptides, soluble proteins, antibodies, polynucleotides, vectors, cells, or compositions are those that have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition that is isolated is substantially pure. A polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition can be isolated from a natural source (e.g., tissue) or from a source such as an engineered cell line.

[0092] The term “substantially pure” as used herein refers to material that is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0093] The term “pharmaceutically acceptable” as used herein refers to a substance approved or approvable by a regulatory agency or listed in the U.S. Pharmacopeia, European Pharmacopeia, or other generally recognized pharmacopeia for use in animals, including humans.

[0094] The terms “pharmaceutically acceptable excipient, carrier, or adjuvant” as used herein refer to an excipient, carrier, or adjuvant that can be administered to a subject, together with at least one antibody of the disclosure, and that is generally safe, non-toxic, and has no effect on the pharmacological activity of the therapeutic agent. In general, those of skill in the art and the U.S. FDA consider a pharmaceutically acceptable excipient, carrier, or adjuvant to be an inactive ingredient of any formulation.

[0095] The term “pharmaceutical composition” as used herein refers to a preparation that is in such form as to permit the biological activity of the antibody to be effective. A pharmaceutical formulation or composition generally comprises additional components, such as a pharmaceutically acceptable excipient, carrier, adjuvant, buffers, etc.

[0096] The term “conjugate” as used herein refers to a combination in which two substances are linked by a covalent bond (e.g., an antibody of the disclosure joined to a

therapeutic agent). In the conjugate, the two substances may be directly connected or may be connected via a linker. In the present disclosure, one of the two substances is an antibody of the disclosure, and the other is a drug (for example, a physiologically active substance). The linker may be a cleavable linker or a non-cleavable linker.

5 **[0097]** The term “effective amount” or “therapeutically effective amount” as used herein refers to an amount of an antibody of the disclosure that is required to reach the tissue of interest, or to an amount of a conjugate, a fusion protein or polypeptide, or a complex comprising an antibody of the disclosure and a therapeutic agent that is sufficient to reduce and/or ameliorate the severity and/or duration of (i) a disease, disorder or condition in a subject, and/or (ii) a
10 symptom in a subject. The term also encompasses an amount of a conjugate necessary for the (i) reduction or amelioration of the advancement or progression of a given disease, disorder, or condition, (ii) reduction or amelioration of the recurrence, development, or onset of a given disease, disorder, or condition, and/or (iii) the improvement or enhancement of the prophylactic or therapeutic effect(s) of another agent or therapy (e.g., an agent other than the conjugates
15 provided herein).

[0098] The term “therapeutic effect” as used herein refers to the effect and/or ability of an agent, e.g., an antibody, a conjugate, a fusion protein or polypeptide, or a complex comprising the antibody of the disclosure to reduce and/or ameliorate the severity and/or duration of (i) a disease, disorder, or condition in a subject, and/or (ii) a symptom in a subject. The term also
20 encompasses the ability of an agent, e.g., a conjugate, to (i) reduce or ameliorate the advancement or progression of a given disease, disorder, or condition, (ii) reduce or ameliorate the recurrence, development, or onset of a given disease, disorder, or condition, and/or (iii) to improve or enhance the prophylactic or therapeutic effect(s) of another agent or therapy (e.g., an agent other than the conjugates provided herein).

25 **[0099]** As used herein, reference to “about” or “approximately” a value or parameter includes (and describes) embodiments that are directed to that value or parameter. For example, a description referring to “about X” includes description of “X”. “About X” means +/- 10% of X. So, “about 10” means a value between 9 to 11.

TFR1 AND ANTI-TFR1 ANTIBODIES

[00100] Transferrin receptor, also known as CD71, is a transmembrane glycoprotein expressed in various sites of the human body at differing levels, whose function is to mediate cellular uptake of iron from a plasma glycoprotein, transferrin. Iron uptake from transferrin involves the binding of transferrin to the transferrin receptor, internalization of transferrin within an endocytic vesicle by receptor-mediated endocytosis and the release of iron from the protein by a decrease in endosomal pH. Ponka P, Lok CN.. Int J Biochem Cell Biol. 1999 Oct;31(10):1111-37 and Xiaopeng Mo, in Brain Targeted Drug Delivery System, 2019. Apotransferrin (i.e., non-iron conjugate) binds to TfR when bound to two Fe 3+ ions to form holotransferrin (i.e., iron conjugate). The complex of TfR and holotransferrin is translocated into the cell by receptor-mediated endocytosis. CD71 and transferrin dissociate in an endosomal environment, and transferrin moves into the cell while CD71 is recycled to the cell membrane. Thus, transferrin is thought to translocate into cells by proper binding to TfR and proper dissociation. The transferrin receptor system has been exploited for delivery of anticancer drugs and proteins, therapeutic genes into malignant cells, and to deliver other therapeutic agents across the blood brain barrier to the brain.

[00101] In humans and cynomolgus monkeys, two transferrin receptors, TfR1 and TfR2 have been characterized. TfR1 is a high affinity ubiquitously expressed receptor while expression of TfR2 is restricted to certain cell types and is unaffected by intracellular iron concentrations. TfR2 binds to transferrin with a 25-30 fold lower affinity than TfR1. The antibodies of the present disclosure bind to TfR1.

[00102] The sequences for human TfR1 and cyno TfR1 are as follows:

Human TfR1 (UniProt No. P02786.2; SEQ ID NO:1)

25 MMDQARSAFSLNFGGEPLSYTRFSLARQVDGDNHVMKLAVDDEENADNNTKANVTKPKRC
SGSICYGTIAVIVFFLIGFMIGYLG YCKGVEPKTECERLAGTESPVREEPGEDFPAARRLYWDDLK
RKLSEKLDSTDFGTIKLLNENSYVPREAGSQKDENLALYVENQFREFKLSKVWRDQHFVKIQV
KDSAQNSVIIVDKNGRLVYLVENPGGYVAYSKAATVTGKL V HANFGTKKDFEDLYTPVNGSIVI
VRAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVNAELSFFGHAHLGTGDPYTPGFPSFNHTQFP
30 PSRSSGLPNIPVQTISRAAAEKLFGNMEGDCPSDWKTDSTCRMVTSSESKNVKLTVSNVLKEIKILN
IFGVIKGFVEPDHYVVVGAQRDAWGPGAAGSGVGTALLKLAQMFSDMVLKDGFPQRSIIFAS
WSAGDFGSVGATEWLEGYLSSLHLKAFTYINLDKAVLGTSNFKVVSAPLLYTLIEKTMQNVKHP
VTGQFLYQDSNWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELIERIP
ELNKVARAAAEVAGQFVIKLTHDVELNLDYERYNSQLLSFVRDLNQYRADIKEMGLSLQWLYS

ARGDFFRATSRLTTDFGNAEKTDRFVMKKLNDRVMRVEYHFLSPYVSPKESPRHVFVWGSGSHT
LPALLENLKLKRNNGAFNETLFRNQLALATWTIQGAANALSGDVWDIDNEF

Cyno TfR1 (UniProt No. G8F602; SEQ ID NO:2)

5 MMDQARSAFSNLFGGEPLSYTRFSLARQVDGDNSHVEMKLAVDDEENADNNTKANGTKPKRC
GGNICYGTIAVHIFLIGFMIGYLG YCKGVEPKTECERLAGTESPAREEPEEDFPAAPRLYWDDLK
RKLSEKLDTTDFSTIKLLNENLYVPREAGSQKDENLALYIENQFREFKLSKVWRDQHFVKIQVK
DSAQNSVIIVDKNGGLVYLVENPGGYVAYSKAATVTGKLVHANFGTKKDFEDLDSPVNGSIVIV
RAGKITFAEKVANAESLNAIGVLIYMDQTKFPIVKADLSFFGHAHLGTGDPYTPGFPSFNHTQFPP
10 SQSSGLPNIPVQTISRAAAEKLFNGMEGDCPSDWKTDSTCKMVTSENKSVKLTVSNVLKETKILN
IFGVIKGFVEPDHYVVVGAQRDAWGPGAAKSSVGTALLKLAQMFSDMVLKDGFPQRSIIFAS
WSAGDFGSGVATEWLEGYLSLHLKAFTYINLDKAVLGTSNFKVSASPLLYTLIEKTMQDVKHP
VTGRSLYQDSNWASKVEKLTLDNAAFPFLAYSGIPAVSFCFCEDTDYPYLGTTMDTYKELVERIP
ELNKVARAAAEVAGQFVIKLTHTDELNLDYERYNSQLLLFLRDLNQYRADVKEMGLSLQWLYS
15 ARGDFFRATSRLTTDFRNAEKRDKFVMKKLNDRVMRVEYYFLSPYVSPKESPRHVFVWGSGSH
TSLALLESLKLRRQNNSAFNETLFRNQLALATWTIQGAANALSGDVWDIDNEF

[00103] The present disclosure provides antibodies that bind TfR1.

[00104] In some embodiments, the anti-TfR1 antibody is a recombinant antibody. In some
20 embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a
chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some
embodiments, the antibody is a human antibody. In some embodiments, the antibody is an IgA,
IgD, IgE, IgG, or IgM antibody. In some embodiments, the antibody is an IgG antibody. In some
embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG2
25 antibody. In some embodiments, the antibody is an IgG3 antibody. In some embodiments, the
antibody is an IgG4 antibody. In some instances, the antibody comprises the human kappa light
chain constant region. In other embodiments, the antibody comprises a human lambda light chain
constant region. In some instances, the antibody is an IgG1 antibody and comprises a human
kappa light chain constant region. In some instances, the antibody is an IgG1 antibody and
30 comprises a human lambda light chain constant region. In some embodiments, the antibody is an
antibody fragment comprising an antigen-binding site. In some embodiments, the antibody is a
scFv. In some embodiments, the antibody is a disulfide-linked scFv. In some embodiments, the
antibody is a bispecific antibody or a multispecific antibody. In some embodiments, the antibody
is a monovalent antibody. In some embodiments, the antibody is a monospecific antibody. In
35 some embodiments, the antibody is a bivalent antibody.

[00105] In some instances, the antibody is an Fab, Fab', F(ab)₂, scFv, sc(Fv)₂, diabody, or
nanobody. In some embodiments, the interchain disulfide in the antibody or antigen binding

fragment (e.g., Fab) is removed. The Fab or Fab' contains a variable heavy (VH) and a variable light domain (VL).

[00106] In some embodiments, the antibody is isolated. In some embodiments, the antibody is substantially pure.

5 **[00107]** In some embodiments, the anti-TfR1 antibody is a humanized antibody. Various methods for generating humanized antibodies are known in the art. In some embodiments, a humanized antibody comprises one or more amino acid residues that have been introduced into its sequence from a source that is non-human. In some embodiments, humanization is performed by substituting one or more non-human CDR sequences for the corresponding CDR sequences of
10 a human antibody.

[00108] The choice of which human heavy chain variable region and/or light chain variable region are used for generating humanized antibodies can be made based on a variety of factors and by a variety of methods known in the art. In some embodiments, the "best-fit" method is used where the sequence of the variable region of a non-human (e.g., rodent) antibody
15 is screened against the entire library of known human variable region sequences. The human sequence that is most similar to that of the non-human (e.g., rodent) sequence is selected as the human variable region framework for the humanized antibody. In some embodiments, a particular variable region framework derived from a consensus sequence of all human antibodies of a particular subgroup of light or heavy chains is selected as the variable region framework. In
20 some embodiments, the variable region framework sequence is derived from the consensus sequences of the most abundant human subclasses. In some embodiments, human germline genes are used as the source of the variable region framework sequences.

[00109] Other methods for humanization include, but are not limited to, (i) a method called "superhumanization" that is described as the direct transfer of CDRs to a human germline
25 framework, (ii) a method termed Human String Content (HSC) that is based on a metric of "antibody humanness", (iii) methods based on generation of large libraries of humanized variants (including phage, ribosomal, and yeast display libraries), and (iv) methods based on framework region shuffling.

[00110] In some embodiments, the anti-TfR1 antibody is a "human antibody". Human
30 antibodies can be prepared using various techniques known in the art. In some embodiments, human antibodies are generated from immortalized human B lymphocytes immunized in vitro.

In some embodiments, human antibodies are generated from lymphocytes isolated from an immunized individual. In any case, cells that produce an antibody directed against a target antigen can be generated and isolated. In some embodiments, a human antibody is selected from a phage library, where that phage library expresses human antibodies. Alternatively, phage display technology may be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable region gene repertoires from unimmunized donors. Techniques for the generation and use of antibody phage libraries are well-known in the art. Once antibodies are identified, affinity maturation strategies known in the art, including but not limited to, chain shuffling and site-directed mutagenesis, may be employed to generate higher affinity human antibodies. In some embodiments, human antibodies are produced in transgenic mice that contain human immunoglobulin loci. Upon immunization these mice are capable of producing the full repertoire of human antibodies in the absence of endogenous immunoglobulin production.

[00111] In some embodiments, the anti-TfR1 antibody is a bispecific antibody. Bispecific antibodies are capable of recognizing and binding at least two different antigens or epitopes. The different epitopes can either be within the same molecule (*e.g.*, two epitopes on TfR1) or on different molecules (*e.g.*, one epitope on TfR1 and one epitope on a different target). In some embodiments, a bispecific antibody has enhanced potency as compared to an individual antibody or to a combination of more than one antibody. In some embodiments, a bispecific antibody has reduced toxicity as compared to an individual antibody or to a combination of more than one antibody. It is known to those of skill in the art that any therapeutic agent may have unique pharmacokinetics (PK) (*e.g.*, circulating half-life). In some embodiments, a bispecific antibody has the ability to synchronize the PK of two active binding agents wherein the two individual binding agents have different PK profiles. In some embodiments, a bispecific antibody has the ability to concentrate the actions of two agents in a common area (*e.g.*, tissue) in a subject. In some embodiments, a bispecific antibody has the ability to concentrate the actions of two agents to a common target (*e.g.*, a specific cell type). In some embodiments, a bispecific antibody has the ability to target the actions of two agents to more than one biological pathway or function. In some embodiments, a bispecific antibody has the ability to target two different cells and bring them closer together.

[00112] In some embodiments, a bispecific antibody has decreased toxicity and/or side effects. In some embodiments, a bispecific antibody has decreased toxicity and/or side effects as compared to a mixture of the two individual antibodies or the antibodies as single agents. In some embodiments, a bispecific antibody has an increased therapeutic index. In some
5 embodiments, a bispecific antibody has an increased therapeutic index as compared to a mixture of the two individual antibodies or the antibodies as single agents.

[00113] Several techniques for making bispecific antibodies are known by those skilled in the art. In some embodiments, the bispecific antibodies comprise heavy chain constant regions with modifications in the amino acids that are part of the interface between the two heavy chains.
10 These modifications are made to enhance heterodimer formation and generally reduce or eliminate homodimer formation. In some embodiments, the bispecific antibodies are generated using a knobs-into-holes (KIH) strategy. See., e.g., Ridgway et al. Protein Eng. 1996;9(7):617-21 and Klein et al. MAbs. 2012;4(6):653-663.

[00114] In some embodiments, the bispecific antibodies comprise light chain constant
15 regions with modifications in the amino acids that are part of the interface between the two light chains. These modifications are made to reduce or eliminate light chain mispairing. See, e.g., Lewis et al. Nat Biotech 2014;32(2):191-98. In some embodiments, the bispecific antibodies comprise an scFv that covalently links the VH and VL and removes CH1 and CL. In some
20 embodiments, the bispecific antibodies comprise an scFab or Fcab (see, e.g., Wozniak-Knopp et al. PEDS 2010;23(4):289-97), single-domain antibodies (e.g., with VHHs from camelid species or sharks), or Duet Mabs (see, e.g., Mazor et al. Mabs 2015;7(2):377-89).

[00115] Bispecific antibodies can be intact antibodies or antibody fragments comprising antigen-binding sites.

[00116] Anti-TfR1 antibodies with more than two specificities are contemplated in this
25 disclosure. In some embodiments, trispecific or tetraspecific antibodies are generated. Anti-TfR1 antibodies with more than two valencies are contemplated. In some embodiments, trivalent or tetravalent antibodies are generated.

[00117] CDRs of an antibody are defined by those skilled in the art using a variety of methods/systems. These systems and/or definitions have been developed and refined over a
30 number of years and include Kabat, Chothia, IMGT, AbM, Contact, and Union. The Kabat definition is based on sequence variability and is commonly used. The Chothia definition is

based on the location of the structural loop regions. The IMGT system is based on sequence variability and location within the structure of the variable domain. The AbM definition is a compromise between Kabat and Chothia. The Contact definition is based on analyses of the available antibody crystal structures. An Exemplary system is a combination of Kabat and Chothia. Software programs (*e.g.*, abYsis) are available and known to those of skill in the art for analysis of antibody sequence and determination of CDRs.

[00118] The CDR sequences described in **Table 1** include the union of all positions in the Union CDR definitions (Kabat, E. A., Wu, T. T., Perry, H. M., Gottesman, K. S. & Foeller, C. (1991). Sequences of Proteins of Immunological Interest, 5th edit. National Institutes of Health, Bethesda, MD) and the Chothia CDR definitions (Chothia, C. & Lesk, A.M. J. Mol. Biol (1987) 196, 901-917) (Chothia, C. et al. Nature (1989) 342, 877-883) (Al-Lazikani, B., Lesk, A.M. & Chothia, C. J. Mol. Biol (1997) 273, 927-948). This "union" definition of the CDRs is also known as the "Wolguy" definition by Bujotzek et al. (Bujotzek A1, Dunbar J, Lipsmeier F, Schäfer W, Antes I, Deane CM, Georges G. (2015) "Prediction of VH-VL domain orientation for antibody variable domain modeling." Proteins Apr;83(4):681-95. doi: 10.1002/prot.24756)). In some embodiments, the CDR definition is based on a combination of Kabat and Chothia definitions (Exemplary system). However, it will be understood that reference to a VH CDR or CDRs and/or a VL CDR or CDRs of a specific antibody will encompass all CDR definitions as known to those of skill in the art. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of the ANTIBODY X parental antibody disclosed herein based on the Wolguy or Union definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of the ANTIBODY X parental antibody disclosed herein based on the Chothia definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of the ANTIBODY X parental antibody disclosed herein based on the Kabat definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of the ANTIBODY X parental antibody disclosed herein based on the AbM definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of the ANTIBODY X parental antibody disclosed herein based on the IMGT definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of the ANTIBODY X parental antibody disclosed herein based on the Contact definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of any ANTIBODY X mutant disclosed herein based on the Wolguy or Union

definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of any ANTIBODY X mutant disclosed herein based on the Chothia definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of any ANTIBODY X mutant disclosed herein based on the Kabat definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of any ANTIBODY X mutant disclosed herein based on the AbM definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of any ANTIBODY X mutant disclosed herein based on the IMGT definition. In one instance, the anti-TfR1 antibody described herein comprises the six CDRs of any ANTIBODY X mutant disclosed herein based on the Contact definition.

[00119] **Table 1** describes the “parental” CDRs (according to the Union definition) of the reference ANTIBODY X antibody as well as mutant CDRs present in ANTIBODY X mutants described herein. Mutant amino acid positions are identified with AHo numbering (Honegger et al., J Mol Biol, 2001, 309(3):657-70). Where a mutant CDR is not present, the variable region comprises the corresponding parental CDR. For instance, for VH-S40A / VL Parental, the VH comprises a mutant VH CDR1 (S40A), parental VH CDR2, and parental VH CDR3, and the VL comprises parental VL CDR1, parental VL CDR2, and parental VL CDR3. In some embodiments, the anti-TfR1 antibody is an anti-TfR1 antibody that comprises at least one of the CDR mutations described in **Table 1** (e.g., 1-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the CDR mutations described in **Table 1**), but otherwise contains the parental CDR sequences described in **Table 1**. In some embodiments, an anti-TfR1 antibody comprises (i) one, two, and/or three mutant VH CDRs described in **Table 1**, and/or (ii) one, two, and/or three mutant VL CDRs described in **Table 1**, with all remaining CDRs selected from the parental CDRs depicted in **Table 1**.

Table 1: Union CDRs for ANTIBODY X Parental and Mutants.

Parental CDRs						
	VH CDR1	VH CDR2	VH CDR3	VL CDR1	VL CDR2	VL CDR3
	GFTFSSYSMN (SEQ ID NO:3)	SISSSSYIYY ADSVKG (SEQ ID NO:4)	KYRAGDFDY (SEQ ID NO:5)	RASQSVSSYLA (SEQ ID NO:6)	GASNRAT (SEQ ID NO:7)	QQSSSPPT (SEQ ID NO:8)
Mutant CDRs						
Mutation	VH CDR1	VH CDR2	VH CDR3	VL CDR1	VL CDR2	VL CDR3
VH-S40A / VL Parental	GFTFSSYAMN (SEQ ID NO:9)					
VH-S60A / VL Parental		SISASSSYIYY ADSVKG (SEQ ID NO:10)				
VH-Y67S / VL Parental		SISSSSSYIYA DSVKG (SEQ ID NO:11)				
VH-Y110S / VL Parental			KSRAGDFDY (SEQ ID NO:12)			
VH-R111S / VL Parental			KYSAGDFDY (SEQ ID NO:13)			
VH-A112Y / VL Parental			KYRYGDFDY (SEQ ID NO:14)			
VH- S60A/Y67S / VL Parental		SISASSSYIYY ADSVKG (SEQ ID NO:25)				
VH- S60A/R111 S / VL Parental		SISASSSYIYY ADSVKG (SEQ ID NO:10)	KYSAGDFDY (SEQ ID NO:13)			
VH Parental / VL-S39N				RASQSVSSNYL A (SEQ ID NO:15)		
VH Parental / VL-Y40N				RASQSVSSNLA (SEQ ID NO:16)		
VH Parental / VL-Y57S					GASNRAT (SEQ ID NO:7)	
VH Parental / VL-N69S					GASSRAT (SEQ ID NO:17)	
VH- S60A / VL-S39N		SISASSSYIYY ADSVKG (SEQ ID NO:10)		RASQSVSSNYL A (SEQ ID NO:15)		
VH- S60A / VL-Y57S		SISASSSYIYY ADSVKG (SEQ ID NO:10)			GASNRAT (SEQ ID NO:7)	

[00120] **Table 2** describes the “parental” CDRs (according to the Kabat definition) of the ANTIBODY X antibody as well as mutant CDRs present in ANTIBODY X mutants described herein. Mutant amino acid positions are identified with AHo numbering. Where a mutant CDR is not present, VH or VL comprises the corresponding parental CDR. For instance, for VH-S40A / VL Parental, the VH comprises a mutant VH CDR1 (S40A), parental VH CDR2, and parental VH CDR3, and the VL comprises parental VL CDR1, parental VL CDR2, and parental VL CDR3. In some embodiments, the anti-TfR1 antibody is an anti-TfR1 antibody that comprises at least one of the CDR mutations described in **Table 2** (e.g., 1-10, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the CDR mutations described in **Table 2**), but otherwise contains the parental CDR sequences described in **Table 2**. In some embodiments, an anti-TfR1 antibody comprises (i) one, two, and/or three mutant VH CDRs described in **Table 2**, and/or (ii) one, two, and/or three mutant VL CDRs described in **Table 2**, with all remaining CDRs selected from the parental CDRs depicted in **Table 2**.

Table 2: Kabat CDRs for ANTIBODY X Parental and Mutants.

Parental CDRs						
	VH CDR1	VH CDR2	VH CDR3	VL CDR1	VL CDR2	VL CDR3
	SYSMN (SEQ ID NO:23)	SISSSSYIYYA DSVKG (SEQ ID NO:4)	KYRAGDFDY (SEQ ID NO:5)	RASQSVSSSYL A (SEQ ID NO:6)	GASNRAT (SEQ ID NO:7)	QQSSSPPT (SEQ ID NO:8)
Mutant CDRs						
Mutation	VH CDR1	VH CDR2	VH CDR3	VL CDR1	VL CDR2	VL CDR3
VH-S40A / VL Parental	SYAMN (SEQ ID NO:24)					
VH-S60A / VL Parental		SISASSSYIYYA DSVKG (SEQ ID NO:10)				
VH-Y67S / VL Parental		SISSSSYIYYA DSVKG (SEQ ID NO:11)				
VH-Y110S / VL Parental			KSRAGDFDY (SEQ ID NO:12)			
VH-R111S / VL Parental			KYSAGDFDY (SEQ ID NO:13)			
VH-A112Y / VL Parental			KYRYGDFDY (SEQ ID NO:14)			
VH- S60A/Y67S / VL Parental		SISASSSYIYYA DSVKG (SEQ ID NO:25)				

VH-S60A/R111S / VL Parental		SISASSSYIYYA DSVKG (SEQ ID NO:10)	KYSAGDFDY (SEQ ID NO:13)			
VH Parental / VL-S39N				RASQSVSSNYLA (SEQ ID NO:15)		
VH Parental / VL-Y40N				RASQSVSSNLA (SEQ ID NO:16)		
VH Parental / VL-Y57S					GASNRAT (SEQ ID NO:7)	
VH Parental / VL-N69S					GASSRAT (SEQ ID NO:17)	
VH- S60A / VL-S39N		SISASSSYIYYA DSVKG (SEQ ID NO:10)		RASQSVSSNYLA (SEQ ID NO:15)		
VH- S60A / VL-Y57S		SISASSSYIYYA DSVKG (SEQ ID NO:10)			GASNRAT (SEQ ID NO:7)	

[00121] In some embodiments, an anti-TfR1 antibody comprises a VH CDR1, CDR2, and CDR3 and/or a light chain variable region CDR1, CDR2, and CDR3 from the ANTIBODY X parental antibody described herein. In some embodiments, an anti-TfR1 antibody comprises a
5 VH CDR1, CDR2, and CDR3 and a VL CDR1, CDR2, and CDR3 from the ANTIBODY X parental antibody described herein. In some embodiments, an anti-TfR1 antibody comprises a humanized version or humanized variant of an ANTIBODY X mutant antibody described herein.

[00122] In some embodiments, an anti-TfR1 antibody comprises a VH comprising VH CDR1, VH CDR2, and VH CDR3, and a VL comprising VL CDR1, VL CDR2, and VL CDR3,
10 wherein: (a) the VH CDR1 comprises the amino acid sequence GFTFSSYX₁MN (SEQ ID NO:18) or the amino acid sequence SYX₁MN (SEQ ID NO:26), wherein X₁ is S or A; (b) the VH CDR2 comprises the amino acid sequence SISX₂SSSX₃IYYADSVKG (SEQ ID NO:19), wherein X₂ is S or A, and wherein X₃ is Y or S; (c) the VH CDR3 comprises the amino acid sequence KX₄X₅X₆GDFDY (SEQ ID NO:20), wherein X₄ is Y or S, wherein X₅ is R or S, and
15 wherein X₆ is A or Y; (d) the VL CDR1 comprises the amino acid sequence RASQSVSSX₇X₈LA (SEQ ID NO:21), wherein X₇ is S or N, and wherein X₈ is Y or N; (e) the VL CDR2 comprises the amino acid sequence GASX₉RAT (SEQ ID NO:22), wherein X₉ is N or S; and (f) the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8). In some embodiments, the antibody is monovalent and has a monovalent affinity (K_D) for hTfR1 of
20 > 10 nM. In some embodiments, the antibody is bivalent and has a monovalent affinity (K_D) of

hTfR1 of >10 nM. In some embodiments, the antibody is bivalent and has a monovalent affinity (K_D) for hTfR1 of >100 nM. In some embodiments, the antibody is bivalent and has a monovalent affinity (K_D) for hTfR1 of >1000 nM. In some embodiments, the antibody is bivalent and has a monovalent affinity (K_D) for hTfR1 of >100 nM or >1000 nM. In some
5 embodiments, the valency and affinity of the antibody are targeted for optimal brain exposure.

[00123] In some embodiments, an anti-TfR1 antibody comprises a VH CDR1, CDR2, and CDR3 and/or a light chain variable region CDR1, CDR2, and CDR3 from an ANTIBODY X mutant antibody described herein. In some embodiments, an anti-TfR1 antibody comprises a VH CDR1, CDR2, and CDR3 and a VL CDR1, CDR2, and CDR3 from an ANTIBODY X mutant
10 antibody described herein. In some embodiments, an anti-TfR1 antibody comprises a humanized version or humanized variant of an ANTIBODY X mutant antibody described herein.

[00124] In some embodiments, the anti-TfR1 antibody is a variant of ANTIBODY X parental antibody or of an ANTIBODY X mutant antibody described herein which comprises one to thirty conservative amino acid substitutions. In some embodiments, a variant of the anti-TfR1 antibody comprises one to twenty-five conservative amino acid substitutions. In some
15 embodiments, a variant of the anti-TfR1 antibody comprises one to twenty conservative amino acid substitutions. In some embodiments, a variant of the anti-TfR1 antibody comprises one to fifteen conservative amino acid substitutions. In some embodiments, a variant of the anti-TfR1 antibody comprises one to ten conservative amino acid substitution(s). In some embodiments, a
20 variant of the anti-TfR1 antibody comprises one to five conservative amino acid substitution(s). In some embodiments, a variant of the anti-TfR1 antibody comprises one to three conservative amino acid substitution(s). In some embodiments, the conservative amino acid substitution(s) is in a CDR of the antibody. In some embodiments, the conservative amino acid substitution(s) is in a CDR of the antibody (e.g., at position VH-S40, VH-S60, VH-Y67, VH-Y110, VH-R111,
25 VH-A112, VL-S39, VL-Y40, and/or VL-N69, positions identified with AHO numbering). In some embodiments, the conservative amino acid substitution(s) is not in a CDR of the antibody. In some embodiments, the conservative amino acid substitution(s) is in a framework region of the antibody (e.g., at position VL-Y57 and/or at position VL-S83, positions identified with AHO numbering).

[00125] **Table 3** describes the heavy chain variable region sequences of ANTIBODY X parental and several ANTIBODY X mutants described herein. **Table 4** describes the light chain

variable region sequences of ANTIBODY X parental and several ANTIBODY X mutants described herein. In both tables, mutant amino acid positions are identified with AHo numbering.

Table 3: Heavy Chain Variable Regions of ANTIBODY X and Mutants.

Parental	SEQ ID NO	Sequence
VH-Parental	100	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVS SISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKY RAGDFDYWGQGTLLTVSS
Mutant Name	SEQ ID NO	Sequence
VH-S40A	101	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYAMNWVRQAPGKGLEWVS SISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKY RAGDFDYWGQGTLLTVSS
VH-S60A	102	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVS SISASSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARK YRAGDFDYWGQGTLLTVSS
VH-Y67S	103	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVS SISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKY RAGDFDYWGQGTLLTVSS
VH-Y110S	104	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVS SISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKS RAGDFDYWGQGTLLTVSS
VH-R111S	105	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVS SISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKY SAGDFDYWGQGTLLTVSS
VH-A112Y	106	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVS SISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKY RYGDFDYWGQGTLLTVSS
VH-S60A/Y67S	107	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVS SISASSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKY RAGDFDYWGQGTLLTVSS
VH-S60A/R111S	108	EVQLVESGGGLVHPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVS SISASSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARK YSAGDFDYWGQGTLLTVSS

5

Table 4: Light Chain Variable Regions of ANTIBODY X and Mutants.

Parental	SEQ ID NO	Sequence
VL-Parental	200	EIVMTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASN RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQQSSSPPTFGGGTKVEIK
Mutant Name	SEQ ID NO	Sequence
VL-S39N	201	EIVMTQSPGTLSPGERATLSCRASQSVSSNYLAWYQQKPGQAPRLLIYGASN RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQQSSSPPTFGGGTKVEIK
VL-Y40N	202	EIVMTQSPGTLSPGERATLSCRASQSVSSNLAWYQQKPGQAPRLLIYGASN RATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQQSSSPPTFGGGTKVEIK
VL-Y57S	203	EIVMTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASNR ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQQSSSPPTFGGGTKVEIK
VL-N69S	204	EIVMTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSR ATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQQSSSPPTFGGGTKVEIK

- [00126] In some embodiments, an anti-TfR1 antibody comprises a VH comprising at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:100. In some embodiments, an anti-TfR1 antibody comprises a VL comprising at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:200. In some embodiments, an anti-TfR1 antibody comprises a VH comprising at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:100 and a VL comprising at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:200. In some embodiments, the variation imparted by the percent identity is not at position Y57 and/or S83 of the ANTIBODY X VL (according to AHo numbering of SEQ ID NO:200). In some embodiments, the VL comprises Y57 (or a conservative substitution thereof, e.g., Y57S) and/or S83 (or a conservative substitution thereof) (according to AHo numbering of SEQ ID NO:200).
- [00127] In some embodiments, an anti-TfR1 antibody comprises a VH comprising at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a sequence set forth in **Table 3** and a VL comprising at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to a sequence set forth in **Table 4**, where the VH and VL is not identical to the parental ANTIBODY X VH and VL sequence. In some embodiments, the variation imparted by the percent identity is not at the mutation position of the sequence set forth in **Table 3** or **Table 4**. For example, in some embodiments, an anti-TfR1 antibody comprises a VH comprising at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:101 and a VL comprising at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:200, where the VH is not identical to the parental ANTIBODY X VH sequence, and wherein the VH comprises an S40A substitution (according to AHo numbering of SEQ ID NO:100). In some embodiments, the variation imparted by the percent identity is not at position Y57 and/or S83 of the ANTIBODY X VL (according to AHo numbering of SEQ ID NO:200). In some embodiments, the VL comprises Y57 (or a conservative substitution thereof, e.g., Y57S) and/or S83 (or a conservative substitution thereof) (according to AHo numbering of SEQ ID NO:200).
- [00128] In some embodiments, an anti-TfR1 antibody comprises a heavy chain variable region comprising an amino acid sequence that has the three VH CDRs of the ANTIBODY X parental antibody described herein and which has at least 75%, 80%, 85%, 90%, 95%, 96%,

97%, 98%, 99%, or 100% identity to SEQ ID NO:100 and a light chain variable region comprising an amino acid sequence that has the three VL CDRs of the ANTIBODY X parental antibody described herein and which has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO:200. In some embodiments, the variation imparted by the percent identity is not at position Y57 and/or S83 of the ANTIBODY X VL (according to AHO numbering of SEQ ID NO:200). In some embodiments, the VL comprises Y57 (or a conservative substitution thereof, e.g., Y57S) and/or S83 (or a conservative substitution thereof) (according to AHO numbering of SEQ ID NO:200).

[00129] In some embodiments, an anti-TfR1 antibody comprises a heavy chain variable region comprising an amino acid sequence that has the three VH CDRs of any ANTIBODY X mutant antibody described herein and which has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of the VH sequences set forth in **Table 3** and a light chain variable region comprising an amino acid sequence that has the three VL CDRs of any ANTIBODY X mutant antibody described herein and which has at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any one of the sequences set forth in **Table 4**. In some embodiments, the variation imparted by the percent identity is not at position Y57 and/or S83 of the ANTIBODY X VL (according to AHO numbering of SEQ ID NO:200). In some embodiments, the VL comprises Y57 (or a conservative substitution thereof, e.g., Y57S) and/or S83 (or a conservative substitution thereof) (according to AHO numbering of SEQ ID NO:200).

[00130] In some embodiments of the anti-TfR1 antibody:

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
5 VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises the amino
10 acid sequence of SEQ ID NO:203;

the VH CDR1 comprises the amino acid sequence GFTFSSYAMN (SEQ ID NO:9); the
VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
15 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
20 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
25 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S
conservative substitution;

30 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the

VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises the amino acid sequence of SEQ ID NO:203;

5 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

10 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:11); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

15 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KSRAGDFDY (SEQ ID NO:12); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

20 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

30 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the

VH CDR3 comprises the amino acid sequence KYRYGDFDY (SEQ ID NO:14); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

5 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSIYYADSVKG (SEQ ID NO:25); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
10 the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
15 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
20 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSIYYADSVKG (SEQ ID NO:4); the
25 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNLA (SEQ ID NO:16); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8); or

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
30 VH CDR2 comprises the amino acid sequence SISSSSIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1

comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASSRAT (SEQ ID NO:17); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8). In some embodiments, the VL comprises Y57 (or a conservative substitution thereof, e.g., Y57S) and/or S83 (or a conservative substitution thereof) (according to AHo numbering of SEQ ID NO:200).

[00131] In some embodiments of the anti-TfR1 antibody:

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8), wherein the VL comprises the amino acid sequence of SEQ ID NO:203;

the VH CDR1 comprises the amino acid sequence SYAMN (SEQ ID NO:24); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises the amino acid sequence of SEQ ID NO:203;

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSSIYYADSVKG (SEQ ID NO:11); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1

comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH
5 CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KSRAGDFDY (SEQ ID NO:12); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

10 the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
15 the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRYGDFDY (SEQ ID NO:14); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
20 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSSYIYYADSVKG (SEQ ID NO:25); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
25 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH
30 CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:16); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8); or

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASSRAT (SEQ ID NO:17); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8). In some embodiments, the VL comprises Y57 (or a conservative substitution thereof, e.g., Y57S) and/or S83 (or a conservative substitution thereof) (according to AHo numbering of SEQ ID NO:200).

[00132] In some embodiments of the anti-TfR1 antibody the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8). In some embodiments, the VL comprises Y57 (or a conservative substitution thereof, e.g., Y57S) and/or S83 (or a conservative substitution thereof) (according to AHo numbering of SEQ ID NO:200).

- [00133]** In some embodiments of the anti-TfR1 antibody the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8). In some embodiments, the VL comprises Y57 (or a conservative substitution thereof, e.g., Y57S) and/or S83 (or a conservative substitution thereof) (according to AHo numbering of SEQ ID NO:200).
- 5
- [00134]** In some embodiments of the anti-TfR1 antibody:
- 10 the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:200;
- the VH comprises the amino acid sequence of SEQ ID NO:101 and the VL comprises the amino acid sequence of SEQ ID NO:200;
- 15 the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO:200;
- the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO:201;
- the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO:203;
- 20 the VH comprises the amino acid sequence of SEQ ID NO:103 and the VL comprises the amino acid sequence of SEQ ID NO:200;
- the VH comprises the amino acid sequence of SEQ ID NO:104 and the VL comprises the amino acid sequence of SEQ ID NO:200;
- 25 the VH comprises the amino acid sequence of SEQ ID NO:105 and the VL comprises the amino acid sequence of SEQ ID NO:200;
- the VH comprises the amino acid sequence of SEQ ID NO:106 and the VL comprises the amino acid sequence of SEQ ID NO:200;
- the VH comprises the amino acid sequence of SEQ ID NO:107 and the VL comprises the amino acid sequence of SEQ ID NO:200;
- 30

the VH comprises the amino acid sequence of SEQ ID NO:108 and the VL comprises the amino acid sequence of SEQ ID NO:200;

the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:201;

5 the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:202;

the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:203; or

10 the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:204.

[00135] In some embodiments, the anti-TfR1 antibody comprises a VH comprising SEQ ID NO:100 and a VL comprising SEQ ID NO:200. In some embodiments, the anti-TfR1 antibody comprises a VH consisting of SEQ ID NO:100 and a VL consisting of SEQ ID NO:200.

15

CONSTANT REGIONS OF ANTI-TFR1 ANTIBODIES

[00136] In some embodiments, the variable region of an anti-TfR1 antibody described herein is fused to a constant region. A constant region has a constant heavy chain (CH) domain (e.g., CH1, hinge, CH2, and/or CH3 domain(s) or any combination thereof) and a constant light chain (CL) domain. In some embodiments, the CH domain is from an IgG1 molecule or an IgG4 molecule. In some embodiments, the CH domain is from an IgG1 molecule. In some embodiments, the CH domain is from an IgG2 molecule, an IgG3 molecule, or an IgG molecule. The VH of an anti-TfR1 antibody described herein can be fused to any one of the following constant heavy chain (CH) constructs as shown in **Table 5** below. The VL of the anti-TfR1 antibody described herein can be fused to any one of the following constant light chain (CL) constructs as shown in **Table 5** below. In some embodiments, the hinge region is any hinge region known in the art. In some embodiments, the hinge region is naturally occurring, e.g., from a naturally occurring IgG1, IgG2, IgG3, or IgG4 molecule. In other embodiments, the hinge region contains modification(s) relative to a naturally occurring hinge.

25
30 **[00137]** In some embodiments, an anti-TfR1 antibody of the disclosure is one in which at least one or more of the constant regions has been modified or deleted. In some embodiments, an

antibody may comprise one or more modifications to the heavy chain constant domain (CH1, CH2 or CH3) and/or to the light chain constant region (CL). In some embodiments, the heavy chain constant region of the modified antibody comprises at least one human constant region. In some embodiments, the heavy chain constant region of the modified antibody comprises more than one human constant region. In some embodiments, the VH is fused to any CH1 construct known in the art, and the VL is fused to any CL known in the art. In some embodiments, the constant light chain (CL) of the constructs is a naturally occurring human Kappa constant region. In some embodiments, modifications to the constant region comprise additions, deletions, or substitutions of one or more amino acids in one or more regions. In some embodiments, one or more regions are partially or entirely deleted from the constant regions of a modified antibody. In some embodiments, the entire CH2 and CH3 domains have been removed from an antibody. In some embodiments, a deleted constant region is replaced by a short amino acid spacer that provides some of the molecular flexibility typically imparted by the absent constant region. In some embodiments, a modified antibody comprises a CH1 domain directly fused to the hinge region of the antibody. In some embodiments, a modified antibody comprises an Fab fused to the bottom of an Fc.

[00138] In some embodiments, an anti-TfR1 antibody of the disclosure contains a linker (e.g., a linker as shown in **Table 5** below). In some embodiments, a linker is positioned between the Fc region and the Fab region of an anti-TfR1 antibody of the disclosure.

Table 5: Constant Region, Hinge Sequences, and Linker Sequences.

Domain	Sequence
IgG1 or IgG4 CH1	
IgG1 CH1 (no hinge) (SEQ ID NO: 300)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKKV
IgG1 CH1 (with hinge underlined) (SEQ ID NO: 301)	ASTKGPSVFPLAPSSKSTSGGTAALG <u>CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVN</u> HKPSNTKVDKKVEPKSCDKTHTCPP
IgG1 CH1 (no hinge) (SEQ ID NO: 302)	ASTKGPSVFPLAPSSKSTSGGTAALG <u>CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVN</u> HKPSNTKVDKRV
IgG1 CH1 (with hinge underlined) (SEQ ID NO: 303)	ASTKGPSVFPLAPSSKSTSGGTAALG <u>CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVN</u> HKPSNTKVDKR VESKYGPPCP

IgG1 CH1 (no hinge) (SEQ ID NO: 304)	ASTKGPSVFPLAPSSKSTSGGTAALG <u>CL</u> VKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVN HKPSNT KVDKKV
IgG1 CH1 (with hinge underlined) (SEQ ID NO: 305)	ASTKGPSVFPLAPSSKSTSGGTAALG <u>CL</u> VKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVN HKPSNT KVDKKVEPKSC
IgG4 CH1 (no hinge) (SEQ ID NO: 306)	ASTKGPSVFPLAPCSRSTSESTAALG <u>CL</u> VKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYT CNV D HKPSNT KVDKRV
IgG4 CH1 (with hinge underlined) (SEQ ID NO: 307)	ASTKGPSVFPLAPCSRSTSESTAALG <u>CL</u> VKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYT CNV D HKPSNT KVDKRVES
Fc hIgG1.agly DelPG (SEQ ID NO: 308)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQD WLNQKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSP
Fc hIgG1.agly DelG (SEQ ID NO: 309)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQD WLNQKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSP
Fc hIgG1.agly (SEQ ID NO: 310)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQD WLNQKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDEL TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG
HingeFc hIgG1.agly (SEQ ID NO: 311)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH HEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQ DWLNQKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG
HingeFc hIgG1.agly(KnobS) (SEQ ID NO: 312)	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH HEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQ DWLNQKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDE LTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG
Fc hIgG1.agly(KnobS) (SEQ ID NO: 313)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQD WLNQKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDEL TKNQVSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG
HingeFc hIgG1.agly(HoleS) (SEQ ID NO: 314)	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSH EDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQD WLNQKEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDEL TKNQVSLSCAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLVSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPG
Hinge region	
IgG1 hinge A (SEQ ID NO: 315)	EPKSCDKTHTCPPCPAPELLGGP
IgG1 hinge B (SEQ ID NO: 316)	EPKSCDKTHTCPP
IgG1 hinge C (SEQ ID NO: 317)	EPKSC
IgG4 hinge D (SEQ ID NO: 318)	ESKYGPPCPPCPAPEFLGGP

IgG4 hinge E (SEQ ID NO: 319)	ESKYGPPCP
IgG4 hinge F	ES
IgG1 hinge G (SEQ ID NO: 320)	EPKS
IgG1 hinge H (SEQ ID NO: 321)	EPKSCD
IgG1 hinge I (SEQ ID NO: 322)	EPKSCDK
IgG1 hinge J (SEQ ID NO: 323)	EPKSCDKT
IgG1 hinge K (SEQ ID NO: 324)	EPKSCDKTH
IgG1 hinge L (SEQ ID NO: 325)	EPKSCDKTHT
IgG1 hinge M (SEQ ID NO: 326)	EPKSCDKTHTC
IgG1 hinge N (SEQ ID NO: 327)	EPKSCDKTHTCP
IgG1 hinge O (SEQ ID NO: 328)	EPKSCDKTHTCPPC
IgG1 hinge P (SEQ ID NO: 329)	EPKSCDKTHTCPPCP
IgG1 hinge Q (SEQ ID NO: 330)	EPKSCDKTHTCPPCPA
IgG1 hinge R (SEQ ID NO: 331)	EPKSCDKTHTCPPCPAP
IgG1 hinge S (SEQ ID NO: 332)	EPKSCDKTHTCPPCPAPE
IgG1 hinge T (SEQ ID NO: 333)	EPKSCDKTHTCPPCPAPEL
IgG1 hinge U (SEQ ID NO: 334)	EPKSCDKTHTCPPCPAPELL
IgG1 hinge V (SEQ ID NO: 335)	EPKSCDKTHTCPPCPAPELLG
IgG1 hinge W (SEQ ID NO: 336)	EPKSCDKTHTCPPCPAPELLGG
IgG4 hinge X	ESK
IgG4 hinge Y (SEQ ID NO: 337)	ESKY
IgG4 hinge Z (SEQ ID NO: 338)	ESKYG
IgG4 hinge ZA (SEQ ID NO: 339)	ESKYGP
IgG4 hinge ZB (SEQ ID NO: 340)	ESKYGPP
IgG4 hinge ZC (SEQ ID NO: 341)	ESKYGPPC
IgG4 hinge ZD (SEQ ID NO: 342)	ESKYGPPCPP
IgG4 hinge ZE (SEQ ID NO: 343)	ESKYGPPCPPC
IgG4 hinge ZF (SEQ ID NO: 344)	ESKYGPPCPPCP
IgG4 hinge ZG (SEQ ID NO: 345)	ESKYGPPCPPCPA
IgG4 hinge ZH (SEQ ID NO: 346)	ESKYGPPCPPCPAP
IgG4 hinge ZI (SEQ ID NO: 347)	ESKYGPPCPPCPAPE
IgG4 hinge ZJ (SEQ ID NO: 348)	ESKYGPPCPPCPAPEF
IgG4 hinge ZK (SEQ ID NO: 349)	ESKYGPPCPPCPAPEFL
IgG4 hinge ZL (SEQ ID NO: 350)	ESKYGPPCPPCPAPEFLG
IgG4 hinge ZM (SEQ ID NO: 351)	ESKYGPPCPPCPAPEFLGG
IgG4 hinge ZN (SEQ ID NO: 352)	ESKYGPPCPPCPAPEFLGGP

CL domain	
Human kappa CL (SEQ ID NO: 353)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC
Human kappa CL (Δ C214) (SEQ ID NO: 354)	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC
Linker	
LinkerS	S
LinkerSG ₂	SGG
LinkerSG ₄ (SEQ ID NO: 355)	SGGGG
LinkerSG ₄ SG ₄ (SEQ ID NO: 356)	SGGGGSGGGG
LinkerSG ₄ SG ₄ SG ₄ (SEQ ID NO: 357)	SGGGGSGGGGSGGGG
LinkerG ₃ SG ₅ (SEQ ID NO: 358)	GGGSGGGGG
LinkerG ₄ SG ₄ SG ₄ S (SEQ ID NO: 359)	GGGGSGGGGSGGGGS
LinkerTG	TG

[00139] Other exemplary constant regions, e.g., hinge regions, that can be combined with the antibody variable regions described herein include but are not limited to the hinge regions described in Peters SJ, et al. J Biol Chem. 2012 Jul 13;287(29):24525-33; and Heads JT, et al. Protein Sci. 2012 Sep;21(9):1315-22; incorporated herein by reference in their entirety.

[00140] In some embodiments, an anti-TfR1 antibody comprises a heavy chain described in Table 6. In some embodiments, an anti-TfR1 antibody comprises a light chain described in Table 6. In some embodiments, an anti-TfR1 antibody comprises a heavy chain described in Table 6 and a light chain described in Table 6.

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Table 6: Heavy Chains and Light Chains of ANTIBODY X and Mutants.

Name	SEQ ID NO	Sequence
ANTIBODY X HC	400	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKYRAGDFDYWGQGTLLTVTSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNK

		ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG
ANTIBODY X LC	401	EIVMTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASNRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQQSSSPFTGGGKVEIKRTVAA PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD SKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
ANTIBODY X Fab fragment HC	402	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSS SYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKYRAGDFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTH
ANTIBODY X Fc-Fab bivalent HC	403	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVR QAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAV YYCARKYRAGDFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSC
ANTIBODY X Fab-Fc monovalent HC1 (knob)	404	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSS SYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARKYRAGDFDYW GQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA LTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK SCDKTHCTPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVK FNWYVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNK ALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPG
ANTIBODY X Fc-Fab monovalent HC1 (knob)	405	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVR QAPGKGLEWVSSISSSSSYIYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAV YYCARKYRAGDFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLV

		KDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNV NHKPSNTKVDKKVEPKSC
Fc (hole)	406	KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALP APIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPG
Anti-BACE1 / ANTIBODY X bivalent HC (constant region)	407	ASTKGPSVFPRAPSSKSTSGGTAALGCLVRDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG GGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGFTSSYSMNWVRQAPGKGL EWVSSISSSSYIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARKY RAGDFDYWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCQVEDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYELSSVTVPSSSLGTQTYICNVNHKPSNT KVDKKVEPKSC
Anti-BACE1 / ANTIBODY X monovalent HC1 (knob) (constant region)	408	ASTKGPSVFPRAPSSKSTSGGTAALGCLVRDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGFTSSYSMNWVRQAPGK GLEWVSSISSSSYIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCAR KYRAGDFDYWGQGTLLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCQVEDYFPE PVTVSWNSGALTSGVHTFPAVLQSSGLYELSSVTVPSSSLGTQTYICNVNHKPSN TKVDKKVEPKSC
Anti-BACE1 / ANTIBODY X monovalent HC2 (hole) (constant region)	409	ASTKGPSVFPRAPSSKSTSGGTAALGCLVRDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESNGQPENNY KTTTPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP G

Anti-BACE1 / ANTIBODY X LC1 (constant region)	410	RTVAAPSVFIFPPSDEELKSGTASVQCLLNIFYPREAKVQWKVDNALQSGNSQES VTEQDSKDYSLSSSELTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
Anti-BACE1 / ANTIBODY X LC2	411	EIVMTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASNRA TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQQSSSPFTGGGKVEIKRTVAA PSVFIFPPSDEQLKSGRASVVCCLLNIFYPREAKVQWKVDNALQSGNSQESVTEQD SKDYSLSSRLQLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
ANTIBODY X S442C HC	412	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSS SYIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARKYRAGDFDYW GQGTLLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKVDKRVESK YGPCCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNW YVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKS LCLSPG
ANTIBODY X monovalent S442C HC1 (knob)	413	EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSS SYIYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARKYRAGDFDYW GQGTLLTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGKTYTCNVDPKPSNTKVDKRVESK YGPCCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFNW YVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLWCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKS LSLSPG
ANTIBODY X monovalent S442C HC2 (hole)	414	KYGPCCPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVDSQEDPEVQFN WYVDGVEVHNAKTKPREEQFQSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGL PSSIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQ KSLCLSPG

[00141] In some instances, an anti-TfR1 antibody comprises a heavy chain and a light chain, wherein the heavy chain comprises or consists of the amino acid sequences set forth in SEQ ID NO:400 and the light chain comprises or consists of the amino acid sequence set forth in

5 SEQ ID NO:401.

[00142] In some instances, an anti-TfR1 antibody comprises a Fab fragment. In some instances, the antibody comprises a Fab fragment, wherein the Fab fragment comprises the amino acid sequences set forth in SEQ ID NOs:401 and 402.

5 [00143] In some instances, an anti-TfR1 antibody comprises a Fab-Fc. In some instances, the antibody comprises a Fab-Fc, wherein the Fab-Fc comprises the amino acid sequences set forth in SEQ ID NOs:401, 404, and 406. In some instances, the antibody comprises a Fab-Fc, wherein the Fab-Fc comprises the amino acid sequences set forth in SEQ ID NOs: 400 and 401.

10 [00144] In some instances, an anti-TfR1 antibody comprises an Fc-Fab. In some instances, the antibody comprises an Fc-Fab, wherein the Fc-Fab comprises the amino acid sequences set forth in SEQ ID NOs:401, 405, and 406. In some instances, the antibody comprises an Fc-Fab, wherein the Fc-Fab comprises the amino acid sequences set forth in SEQ ID NOs: 401 and 403.

[00145] In some embodiments, an anti-TfR1 antibody comprises a heavy chain having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a heavy chain sequence identified herein, wherein the anti-TfR1 antibody has a VH and VL identical to the VH and VL of ANTIBODY X. In some embodiments, an anti-TfR1 antibody comprises a light chain having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a light chain sequence identified herein, wherein the anti-TfR1 antibody has a VH and VL identical to the VH and VL of ANTIBODY X. In some embodiments, an anti-TfR1 antibody comprises a heavy chain having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a heavy chain sequence identified herein and a light chain having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a light chain sequence identified herein, wherein the anti-TfR1 antibody has a VH and VL identical to the VH and VL of ANTIBODY X.

25 [00146] In some embodiments, an anti-TfR1 antibody comprises a heavy chain having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a heavy chain sequence identified herein, wherein the anti-TfR1 antibody does not have a VH and VL identical to the VH and VL of ANTIBODY X. In some embodiments, an anti-TfR1 antibody comprises a light chain having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a light chain sequence identified herein, wherein the anti-TfR1 antibody does not have a VH and VL identical to the VH and VL of ANTIBODY X. In some embodiments, an anti-TfR1 antibody comprises a heavy chain having at least 80%, at least 85%, at least 90%, or at least 95%

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sequence identity to a heavy chain sequence identified herein and a light chain having at least 80%, at least 85%, at least 90%, or at least 95% sequence identity to a light chain sequence identified herein, wherein the anti-TfR1 antibody does not have a VH and VL identical to the VH and VL of ANTIBODY X.

5 **[00147]** In some embodiments, an anti-TfR1 antibody comprises a heavy chain having an amino acid sequence that has one, two, or three, or more modifications (e.g., substitutions, deletions, or insertions) to a heavy chain sequence identified herein, wherein the anti-TfR1 antibody has a VH and VL identical to the VH and VL of ANTIBODY X. In some
10 embodiments, an anti-TfR1 antibody comprises a light chain having an amino acid sequence that has one, two, or three, or more modifications (e.g., substitutions, deletions, or insertions) to a light chain sequence identified herein, wherein the anti-TfR1 antibody has a VH and VL identical to the VH and VL of ANTIBODY X. In some embodiments, an anti-TfR1 antibody comprises a heavy chain having an amino acid sequence that has one, two, or three, or more
15 modifications (e.g., substitutions, deletions, or insertions) to a heavy chain sequence identified herein and a light chain having an amino acid sequence that has one, two, or three, or more modifications (e.g., substitutions, deletions, or insertions) to a light chain sequence identified herein, wherein the anti-TfR1 antibody has a VH and VL identical to the VH and VL of ANTIBODY X.

20 **[00148]** In some embodiments, an anti-TfR1 antibody comprises a heavy chain having an amino acid sequence that has one, two, or three, or more modifications (e.g., substitutions, deletions, or insertions) to a heavy chain sequence identified herein, wherein the anti-TfR1 antibody does not have a VH and VL identical to the VH and VL of ANTIBODY X. In some
25 embodiments, an anti-TfR1 antibody comprises a light chain having an amino acid sequence that has one, two, or three, or more modifications (e.g., substitutions, deletions, or insertions) to a light chain sequence identified herein, wherein the anti-TfR1 antibody does not have a VH and VL identical to the VH and VL of ANTIBODY X. In some embodiments, an anti-TfR1 antibody comprises a heavy chain having an amino acid sequence that has one, two, or three, or more
30 modifications (e.g., substitutions, deletions, or insertions) to a heavy chain sequence identified herein and a light chain having an amino acid sequence that has one, two, or three, or more modifications (e.g., substitutions, deletions, or insertions) to a light chain sequence identified

herein, wherein the anti-TfR1 antibody does not have a VH and VL identical to the VH and VL of ANTIBODY X.

[00149] As described in Example 3, the ANTIBODY X discontinuous epitope is formed by residues Q285, T286, K287, P289, E343, D352, C353, P354, S355, K358, D360, S361, R364 from the apical domain and S492, D560, T561, and R579 from the protease-like domain of hu TfR1 (SEQ ID NO:1). In some embodiments, the anti-TfR1 antibody binds to the same epitope as ANTIBODY X (see Example 3). In some embodiments, the anti-TfR1 antibody binds to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17) amino acids of the ANTIBODY X epitope (e.g., one or more of residues Q285, T286, K287, P289, E343, D352, C353, P354, S355, K358, D360, S361, R364, S492, D560, T561, and R579 of hu TfR1 (SEQ ID NO:1)). In some embodiments, the anti-TfR1 antibody binds to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17) amino acids of the ANTIBODY X epitope (e.g., one or more of residues T286, K287, E343, D352, C353, P354, S355, K358, D360, S361, R364, S492, D560, T561, and R579 of hu TfR1 (SEQ ID NO:1)). In some embodiments, the antibody is monovalent and has a monovalent affinity (K_D) for hTfR1 of >10 nM. In some embodiments, the antibody is bivalent and has a monovalent affinity (K_D) of >10 nM. In some embodiments, the antibody is bivalent and has a monovalent affinity (K_D) for hTfR1 of >100 nM. In some embodiments, the antibody is bivalent and has a monovalent affinity (K_D) for hTfR1 of >1000 nM. In some embodiments, the antibody is bivalent and has a monovalent affinity (K_D) for hTfR1 of >100 nM or >1000 nM. In some embodiments, the valency and affinity of the antibody are targeted for optimal brain exposure.

[00150] The present disclosure further embraces additional variants and equivalents that are substantially homologous to the recombinant, monoclonal, chimeric, humanized, and human antibodies, or antibody fragments thereof, described herein. In some embodiments, it is desirable to modulate the binding affinity of the antibody. In some embodiments, it is desirable to modulate biological properties of the antibody, including but not limited to, specificity, thermostability, expression level, effector function(s), glycosylation, immunogenicity, and/or solubility. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of an antibody, such as changing the number or position of glycosylation sites or altering membrane anchoring characteristics.

[00151] Effector function of antibodies can be modulated by amino acid mutations and/or domain substitutions (e.g., including but not limited to those described in Dumet et al. MABS 2019; 11(8):1341-50, and those described in Wilkinson et al., 2021, PLoS One. 16(12):e0260954, e.g., any of the amino acid alterations in Table 4 therein, e.g., the “LALA”
5 double mutation (human IgG1 L234A/L235A) and the “LALAPG” triple mutation (human IgG1 L234A/L235A/P329G)). Additional characteristics such as pharmacokinetics (e.g., Dall’acqua et al J of Immunology 2002;169 (9) 5171-80), glycosylation, immunogenicity, solubility, and stability can be engineered by modification of Fc by mutations or substitutions. In addition, novel antigen specificity can be engineered into constant domains to create new paratopes (e.g.,
10 Wozniak-Knopp et al. PEDS 2010;23(4):289-97). The affinity or avidity of a Fab may be modulated by changing the linkages between domains of antibodies such as removing the Fab from the top portion of the antibody and linking the Fab to the Fc C-terminus by a linker of any length from zero to 40 amino acids and fusing into the N-terminus of either the VH or VL domain of the Fab creating an “upside-down” antibody with potentially modulated affinity or
15 avidity for binding to antigen, and modulated effector function (e.g., Weber et al. Cell Reports 2018;22:149-62).

[00152] Variations may be a substitution, deletion, or insertion of one or more nucleotides encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the native antibody or polypeptide sequence. In some embodiments, amino acid
20 substitutions are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, e.g., conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. In some embodiments, the substitution, deletion, or insertion includes less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino
25 acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the parent molecule. In some embodiments, variations in the amino acid sequence that are biologically useful and/or relevant may be determined by systematically making insertions, deletions, or substitutions in the sequence and testing the resulting variant
30 proteins for activity as compared to the parent protein.

[00153] In some embodiments, variants may include addition of amino acid residues at the amino- and/or carboxyl-terminal end of the antibody or polypeptide. The length of additional amino acids residues may range from one residue to a hundred or more residues. In some embodiments, a variant comprises an N-terminal methionyl residue. In some embodiments, the variant comprises an additional polypeptide/protein (e.g., Fc region) to create a fusion protein. In some embodiments, a variant is engineered to be detectable and may comprise a detectable label and/or protein (e.g., a fluorescent tag or an enzyme).

[00154] In some embodiments, a cysteine residue not involved in maintaining the proper conformation of an antibody is substituted or deleted to modulate the antibody's characteristics, for example, to improve oxidative stability and/or prevent aberrant disulfide crosslinking and/or facilitate attachment of an agent. Conversely, in some embodiments, one or more cysteine residues are added to create disulfide bond(s) to improve stability.

[00155] In some embodiments, an antibody of the present disclosure comprises variant hinge regions incapable of forming disulfide linkages between identical heavy chains (e.g., reduce homodimer formation). In some embodiments, the antibodies comprise heavy chains with changes in amino acids that result in altered electrostatic interactions. In some embodiments, the antibodies comprise heavy chains with changes in amino acids that result in altered hydrophobic/hydrophilic interactions.

[00156] In some embodiments, an antibody of the present disclosure is "deimmunized". The deimmunization of antibodies generally consists of introducing specific amino acid mutations (e.g., substitutions, deletions, additions) that result in removal of predicted T-cell epitopes without significantly reducing the binding affinity or other desired characteristics of the antibody.

[00157] In some embodiments, an anti-TfR1 antibody described herein has one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) modifications to enable attachment of the anti-TfR1 antibody to an agent as described below.

[00158] The variant antibodies or polypeptides described herein may be generated using methods known in the art, including but not limited to, site-directed mutagenesis, alanine scanning mutagenesis, and PCR mutagenesis.

[00159] In some embodiments an anti-TfR1 antibody described herein is chemically modified. In some embodiments, the anti-TfR1 antibody has been chemically modified by

glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, and/or linkage to a cellular ligand or other protein. Any of numerous chemical modifications may be carried out by known techniques.

[00160] Generally speaking, antigen-antibody interactions are non-covalent and

5 reversible, formed by a combination of hydrogen bonds, hydrophobic interactions, electrostatic and van der Waals forces. When describing the strength of an antigen-antibody complex, the terms affinity and/or avidity are often used. The binding of an antibody to its antigen is a reversible process, and the affinity of the binding is typically reported as an equilibrium dissociation constant (K_D). K_D is the ratio of an antibody dissociation rate (k_{off} , also referred to
10 herein as k_d) (how quickly it dissociates from its antigen) to the antibody association rate (k_{on}) (how quickly it binds to its antigen). In some embodiments, K_D values are determined by measuring the k_{on} and k_{off} rates of a specific antibody/antigen interaction and then using a ratio of these values to calculate the K_D value. In some embodiments, K_D values are used to evaluate and rank the strength of individual antibody/antigen interactions. The lower the K_D of an
15 antibody, the higher the affinity of the antibody for its target. In some embodiments, affinity is measured using SPR technology in a Biacore system. Avidity gives a measure of the overall strength of an antibody-antigen complex. It is dependent on three major parameters: (i) affinity of the antibody for the target, (ii) valency of both the antibody and antigen, and (iii) structural arrangement of the parts that interact.

20 **[00161]** In some embodiments, an anti-TfR1 antibody described herein has a monovalent affinity (K_D) for hTfR1 of > 10 nM (e.g., >25 nM, >50 nM, > 100 nM, >500 nM, >5000 nM, >1 μ M, >2 μ M, >10 nM to 100 nM, >10 nM to 1000 nM, >10 nM to 2000 nM, >10 nM to 5000 nM, >10 nM to 10000 nM, 100 nM to 1000 nM, 100 nM to 5000 nM, 100 nM to 5000 nM, 100 nM to 500 nM, 500 nM to 1 μ M, 50 nM to 1 μ M, 100 nM to 1 μ M, 500 nM to 10 μ M, or 1 μ M to 10
25 μ M) and/or an off rate (k_d) of $\geq 0.01/s$. In some embodiments, the anti-TfR1 antibody is monovalent or multivalent (e.g., bivalent). In some embodiments, an anti-TfR1 antibody described herein is a multivalent (e.g., bivalent) antibody and has a monovalent affinity of >100 nM. In some embodiments, an anti-TfR1 antibody described herein is a multivalent (e.g., bivalent) antibody and has a monovalent affinity of >1000 nM. In some embodiments, an anti-
30 TfR1 antibody described herein is a multivalent (e.g., bivalent) antibody and has a monovalent affinity of 100 nM to 1000 nM. In some embodiments, an anti-TfR1 antibody described herein

is a multivalent (e.g., bivalent) antibody and has a monovalent affinity of 100 nM to 10000 nM. In some embodiments, an anti-TfR1 antibody described herein is a multivalent (e.g., bivalent) antibody and has a monovalent affinity of 100 nM to 5000 nM. In some embodiments, an anti-TfR1 antibody described herein is a monovalent antibody and has a monovalent affinity for hTfR1 of > 10 nM.

METHODS OF MAKING ANTI-TFR1 ANTIBODIES

[00162] The anti-TfR1 antibodies described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods to constructing a DNA sequence encoding polypeptide sequences and expressing those sequences in a suitable host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional variants thereof. In some embodiments, a DNA sequence encoding a polypeptide of interest is constructed by chemical synthesis using an oligonucleotide synthesizer. Oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize a polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[00163] Once assembled (by synthesis, site-directed mutagenesis, or another method), a polynucleotide sequence encoding a particular polypeptide of interest can be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction enzyme mapping, and/or expression of a biologically active polypeptide in a suitable host. As is well-known in the art, in order to obtain high expression levels of a

transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[00164] In some embodiments, a recombinant expression vector is used to amplify and express DNA encoding an antibody against human TfR1. For example, a recombinant expression vector can be a replicable DNA construct that includes synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of an anti-TfR1 antibody operatively linked to suitable transcriptional and/or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence that is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Regulatory elements can include an operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can also be included. DNA regions are “operatively linked” when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor that participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. In some embodiments, structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. In some embodiments, in situations where recombinant protein is expressed without a leader or transport sequence, a polypeptide may include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

[00165] The choice of an expression control sequence and an expression vector generally depends upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial

plasmids, such as plasmids from *E. coli*, including pCR1, pBR322, pMB9 and their derivatives, and wider host range plasmids, such as M13 and other filamentous single-stranded DNA phages.

[00166] In some embodiments, an anti-TfR1 antibody of the present disclosure is expressed from one or more vectors. In some embodiments, a heavy chain polypeptide is expressed by one vector and a light chain polypeptide is expressed by a second vector. In some embodiments, a heavy chain polypeptide and a light chain polypeptide are expressed by one vector. Thus, the present disclosure provides vectors encoding an anti-TfR1 antibody described herein. In one embodiment, the vector encodes a heavy chain polypeptide of an anti-TfR1 antibody described herein. In one embodiment, the vector encodes a light chain polypeptide of an anti-TfR1 antibody described herein. In one embodiment, the vector encodes a heavy chain polypeptide and a light chain polypeptide of an anti-TfR1 antibody described herein.

[00167] Suitable host cells for expression of an anti-TfR1 antibody or a TfR1 protein or fragment thereof to use as an antigen or immunogen include prokaryotes, yeast cells, insect cells, or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram-negative or gram-positive organisms, for example *E. coli* or *Bacillus*. Higher eukaryotic cells include established cell lines of mammalian origin as described herein. Cell-free translation systems may also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts, as well as methods of protein production, including antibody production are well-known in the art.

[00168] Various mammalian culture systems may be used to express recombinant polypeptides. Expression of recombinant proteins in mammalian cells may be desirable because these proteins are generally correctly folded, appropriately modified, and biologically functional. Examples of suitable mammalian host cell lines include, but are not limited to, COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants thereof. Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

[00169] Expression of recombinant proteins in insect cell culture systems (*e.g.*, baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art.

5 [00170] Thus, the present disclosure provides cells comprising the anti-TfR1 antibody described herein. The present disclosure also provides cells comprising one or more polynucleotides encoding an anti-TfR1 antibody described herein or one or more vectors encoding anti-TfR1 antibody described herein. In one embodiment, the cell comprises a polynucleotide encoding an anti-TfR1 antibody described herein. In one embodiment, the cell
10 comprises a first polynucleotide encoding a heavy chain of an anti-TfR1 antibody described herein and a second polynucleotide encoding a light chain of an anti-TfR1 antibody described herein. In one embodiment, the cell comprises a polynucleotide encoding a heavy chain and a light chain of an anti-TfR1 antibody described herein. In one embodiment, the cell comprises a vector encoding an anti-TfR1 antibody described herein. In one embodiment, the cell
15 comprises a first vector encoding a heavy chain of an anti-TfR1 antibody described herein and a second vector encoding a light chain of an anti-TfR1 antibody described herein. In one embodiment, the cell comprises a vector encoding a heavy chain and a light chain of an anti-TfR1 antibody described herein. In some embodiments, the cells produce the anti-TfR1 antibodies described herein. In some embodiments, the cells produce an antibody. In some
20 embodiments, the cells produce an antibody that binds human TfR1. In some embodiments, the cells produce an antibody that binds cyno TfR1. In some embodiments, the cells produce an antibody that binds human TfR1 and cyno TfR1. In some embodiments, the cell is a prokaryotic cell (*e.g.*, *E. coli*). In some embodiments, the cell is a eukaryotic cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a hybridoma cell.

25 [00171] Proteins produced by a host cell can be purified according to any suitable method. Standard methods include chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexa-histidine (SEQ ID NO:360), maltose binding domain, influenza coat sequence, and glutathione-S-transferase can be attached to the protein to
30 allow easy purification by passage over an appropriate affinity column. Affinity chromatography used for purifying immunoglobulins include, but are not limited to, Protein A,

Protein G, and Protein L chromatography. Isolated proteins can be physically characterized using techniques known to those of skill in the art, including but not limited to, proteolysis, size exclusion chromatography (SEC), mass spectrometry (MS), nuclear magnetic resonance (NMR), isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and x-ray
5 crystallography. The purity of isolated proteins can be determined using techniques known to those of skill in the art, including but not limited to, SDS-PAGE, SEC, capillary gel electrophoresis, IEF, and capillary isoelectric focusing (cIEF).

[00172] In some embodiments, supernatants from expression systems that secrete recombinant protein into culture media are first concentrated using a commercially available
10 protein concentration filter, for example, an Amicon® or Millipore Pellicon® ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. In some embodiments, an anion exchange resin is employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. In some
15 embodiments, a cation exchange step is employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In some embodiments, a hydroxyapatite media is employed, including but not limited to, ceramic hydroxyapatite (CHT). In some embodiments, one or more reverse-phase HPLC steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, are employed to
20 further purify a recombinant protein. In some embodiments, hydrophobic interaction chromatography (HIC) is used to separate recombinant proteins based on their hydrophobicity. HIC is a useful separation technique for purifying proteins while maintaining biological activity due to the use of conditions and matrices that operate under less denaturing conditions than some other techniques. Some or all of the foregoing purification steps, in various combinations, can be
25 employed to provide a homogeneous recombinant protein.

[00173] In some embodiments, the antibody of this disclosure is an Fab that can be generated by first making a full monoclonal Ab followed by digesting the monoclonal antibody by chemical or enzymatic cleavage (*e.g.*, pepsin, papain, or ficin digestion) to yield a F(ab')₂ fragment, followed by reduction of those fragments to yield Fab fragments. Such techniques are
30 known in the art. See, *e.g.*, Victor C-G et al., *Biosensors and Bioelectronics*, 2016 (85):32-45.

[00174] Alternatively, the antibody of this disclosure is made by recombinant synthesis of F(ab')₂ antibody fragments, followed by chemical reduction of these fragments to yield Fab units.

5 POLYNUCLEOTIDES

[00175] In some embodiments, the disclosure encompasses polynucleotides comprising polynucleotides that encode a polypeptide (*e.g.*, an anti-TfR1 antibody) described herein. The term "polynucleotides that encode a polypeptide" encompasses a polynucleotide that includes only coding sequences for the polypeptide as well as a polynucleotide that includes additional
10 coding and/or non-coding sequences. The polynucleotides of the disclosure can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or single-stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand. In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a nucleotide sequence) encoding a heavy chain of an anti-TfR1 antibody
15 described herein. In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a nucleotide sequence) encoding a light chain of an anti-TfR1 antibody described herein. In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a nucleotide sequence) encoding a heavy chain of an anti-TfR1 antibody described herein and a polynucleotide (*e.g.*, a nucleotide sequence) encoding a light chain of an anti-TfR1 antibody.

20 [00176] In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a nucleotide sequence) encoding a polypeptide comprising a VH amino acid sequence depicted in **Table 3**. In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a nucleotide sequence) encoding a polypeptide comprising a VL amino acid sequence depicted in **Table 4**. In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a
25 nucleotide sequence) encoding a polypeptide comprising a VH amino acid sequence depicted in **Table 3** and a polypeptide comprising a VL amino acid sequence depicted in **Table 4**. In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a nucleotide sequence) encoding a polypeptide comprising a heavy chain amino acid sequence depicted in **Table 6**. In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a nucleotide sequence)
30 encoding a polypeptide comprising a light chain amino acid sequence depicted in **Table 6**. In some embodiments, the polynucleotide comprises a polynucleotide (*e.g.*, a nucleotide sequence)

encoding a polypeptide comprising a heavy chain amino acid sequence depicted in **Table 6** and a polypeptide comprising a light chain amino acid sequence depicted in **Table 6**.

[00177] The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some embodiments, a polynucleotide variant contains alterations that produce silent substitutions, additions, or deletions, but does not alter the properties or activities of the encoded polypeptide. In some embodiments, a polynucleotide variant comprises silent substitutions that results in no change to the amino acid sequence of the polypeptide (due to the degeneracy of the genetic code). In some embodiments, a polynucleotide variant comprises one or more mutated codons comprising one or more (e.g., 1, 2, or 3) substitutions to the codon that change the amino acid encoded by that codon. Methods for introducing one or more substitutions into a codon are known in the art, such as, e.g., PCR mutagenesis and site-directed mutagenesis. Polynucleotide variants can be produced for a variety of reasons, for example, to optimize codon expression for a particular host (e.g., change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*). In some embodiments, a polynucleotide variant comprises at least one silent mutation in a non-coding or a coding region of the sequence.

[00178] In some embodiments, a polynucleotide variant is produced to modulate or alter expression (or expression levels) of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to increase expression of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to decrease expression of the encoded polypeptide. In some embodiments, a polynucleotide variant has increased expression of the encoded polypeptide as compared to a parental polynucleotide sequence. In some embodiments, a polynucleotide variant has decreased expression of the encoded polypeptide as compared to a parental polynucleotide sequence.

[00179] In some embodiments, a polynucleotide comprises the coding sequence for a polypeptide (e.g., an antibody) fused in the same reading frame to a polynucleotide that aids in expression and secretion of a polypeptide from a host cell (e.g., a leader sequence that functions as a secretory sequence for controlling transport of a polypeptide). The polypeptide can have the leader sequence cleaved by the host cell to form a “mature” form of the polypeptide.

[00180] In some embodiments, a polynucleotide comprises the coding sequence for a polypeptide (e.g., an antibody) fused in the same reading frame to a marker or tag sequence. For example, in some embodiments, a marker sequence is a hexa-histidine (SEQ ID NO:360) tag

(HIS-tag) that allows for efficient purification of the polypeptide fused to the marker. In some embodiments, a marker sequence is a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (*e.g.*, COS-7 cells) is used. In some embodiments, the marker sequence is a FLAG™ tag. In some embodiments, a marker is used in conjunction with other markers or tags.

[00181] In some embodiments, the polynucleotides are isolated. In some embodiments, the polynucleotides are substantially pure.

VECTORS AND CELLS

[00182] Vectors and cells comprising each and every one of the polynucleotides described herein are also provided. In some embodiments, an expression vector comprises a polynucleotide molecule encoding an anti-TfR1 antibody described herein. In some embodiments, an expression vector comprises a polynucleotide molecule encoding a polypeptide that is part of an anti-TfR1 antibody described herein. In some embodiments, an expression vector comprises a polynucleotide molecule encoding a heavy chain polypeptide of an anti-TfR1 antibody described herein. In some embodiments, an expression vector comprises a polynucleotide molecule encoding a light chain polypeptide of an anti-TfR1 antibody described herein. In some embodiments, an expression vector comprises a polynucleotide molecule encoding a heavy chain polypeptide and a light chain polypeptide of anti-TfR1 antibody described herein. In some embodiments, a host cell comprises an expression vector comprising the polynucleotide molecule encoding an anti-TfR1 antibody described herein. In some embodiments, a host cell comprises an expression vector comprising the polynucleotide molecule encoding a polypeptide that is part of an anti-TfR1 antibody described herein. In some embodiments, a host cell comprises a polynucleotide molecule encoding an anti-TfR1 antibody described herein. In some embodiments, a host cell comprises an expression vector comprising a polynucleotide molecule encoding a heavy chain polypeptide of an anti-TfR1 antibody described herein. In some embodiments, a host cell comprises an expression vector comprising a polynucleotide molecule encoding a light chain polypeptide of an anti-TfR1 antibody described herein. In some embodiments, a host cell comprises an expression vector comprising a first polynucleotide encoding a heavy chain polypeptide and a second polynucleotide light chain polypeptide of an anti-TfR1 antibody described herein. In some embodiments, a host cell

comprises: (ii) a first expression vector comprising a polynucleotide molecule encoding a heavy chain polypeptide of an anti-TfR1 antibody described herein, and (ii) a second expression vector comprising a polynucleotide molecule encoding a light chain polypeptide of the anti-TfR1 antibody.

5

ANALYSIS OF PHYSICAL/CHEMICAL PROPERTIES OF ANTI-TFR1 ANTIBODIES

[00183] Anti-TfR1 antibodies of the present disclosure may be analyzed for their physical/chemical properties and/or biological activities by various methods known in the art. In some embodiments, an anti-TfR1 antibody is tested for its ability to bind TfR1 (*e.g.*, human TfR1 and/or cyno TfR1). Binding assays include, but are not limited to, SPR (*e.g.*, Biacore), ELISA, and flow cytometry. In some embodiments, an anti-TfR1 antibody is tested for its ability to inhibit, reduce, or block binding of transferrin to its TfR1 receptor. In some embodiments, an anti-TfR1 antibody is tested for its ability to inhibit, reduce, or block TfR1 activity. In some embodiments, an anti-TfR1 antibody is tested for its ability to internalize with TfR1 and induce increased internalization of TfR1. In addition, antibodies may be evaluated for solubility, stability, thermostability, viscosity, expression levels, expression quality, and/or purification efficiency.

[00184] In some embodiments, assays are provided for identifying an anti-TfR1 antibody that affects TfR1 activity. In some embodiments, SPR, ELISA, or FACS assays are used to assess the ability of an anti-TfR1 antibody to block binding of TfR1 to Tf. In some embodiments, cytotoxicity assays are used to assess the ability of an anti-TfR1 antibody to affect natural killer (NK) cell activity. In some embodiments, proliferation assays are used to assess the ability of an anti-TfR1 antibody to affect T-cell activity.

[00185] In some embodiments, an anti-TfR1 antibody described herein is an antagonist of human TfR1. In some instances, the terms “inhibiting”, “inducing”, “reducing”, “increasing”, “enhancing” are relative to levels/activity in the absence of treatment with a conjugate comprising the anti-TfR1 antibody. In some instances, the terms “inhibiting”, “inducing”, “reducing”, “increasing”, “enhancing” are relative to levels/activity prior to treatment with a conjugate comprising the anti-TfR1 antibody.

ANTI-TFR1 ANTIBODY CONJUGATES AND COMPLEXES

[00186] The present disclosure also provides conjugates comprising an anti-TfR1 antibody described herein conjugated to a second molecule. In some embodiments, the second molecule comprises any agent, e.g., therapeutic agent, described herein.

5 **[00187]** Conjugates comprising an anti-TfR1 antibody described herein may be made using any suitable method known in the art. In some embodiments, the components of the conjugate are linked by covalent interactions. In some embodiments, conjugates are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

15 **[00188]** In some embodiments, a free cysteine is introduced into the constant region of an anti-TfR1 antibody described herein to facilitate conjugation to an agent. Suitable free cysteine substitutions are known in the art, such as, e.g., those described in Zhou et al., *Pharmaceuticals*. 2021, 4(7):672 (see, e.g., Table 1), which is incorporated by reference herein in its entirety. In some embodiments, an anti-TfR1 antibody described herein comprises an S442C substitution
20 (EU numbering; Stimmel et al., *JBC*. 275(39):30445-30450), which facilitates maleimide-based conjugation of an agent, e.g., an ASO.

[00189] In some embodiments, the components of the conjugate are linked by a method described in Ohri et al., *Bioconjugate Chem.* 2018, 29, 473-485, Yamazoe et al., *Bioconjugate Chemistry* 2020 31 (4), 1199-1208, Shen et al., *Nat Biotechnol.* 2012 Jan 22;30(2):184-9, Dimasi et al., *Mol. Pharmaceutics* 2017, 14, 1501-1516, Stimmel et al., *JBC*. 275(39):30445-30450, and
25 Sussman et al., *Protein Engineering, Design & Selection*, 2017, 31(2):47-54, each of which is incorporated by reference herein in its entirety.

[00190] In some embodiments, an anti-TfR1 antibody described herein is conjugated to a detectable substance or molecule that allows the agent to be used for diagnosis and/or detection.
30 A detectable substance can include, but is not limited to, enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-galactosidase, and acetylcholinesterase; prosthetic groups,

such as biotin and flavine(s); fluorescent materials, such as, umbelliferone, fluorescein, fluorescein isothiocyanate (FITC), rhodamine, tetramethylrhodamine isothiocyanate (TRITC), dichlorotriazinylamine fluorescein, dansyl chloride, cyanine (Cy3), and phycoerythrin; bioluminescent materials, such as luciferase; radioactive materials, such as ^{212}Bi , ^{14}C , ^{57}Co , ^{51}Cr , ^{67}Cu , ^{18}F , ^{68}Ga , ^{67}Ga , ^{153}Gd , ^{159}Gd , ^{68}Ge , ^3H , ^{166}Ho , ^{131}I , ^{125}I , ^{123}I , ^{121}I , ^{115}In , ^{113}In , ^{112}In , ^{111}In , ^{140}La , ^{177}Lu , ^{54}Mn , ^{99}Mo , ^{32}P , ^{103}Pd , ^{149}Pm , ^{142}Pr , ^{186}Re , ^{188}Re , ^{105}Rh , ^{97}Ru , ^{35}S , ^{47}Sc , ^{75}Se , ^{153}Sm , ^{113}Sn , ^{117}Sn , ^{85}Sr , $^{99\text{m}}\text{Tc}$, ^{201}Ti , ^{133}Xe , ^{90}Y , ^{69}Yb , ^{175}Yb , ^{65}Zn ; positron emitting metals; and magnetic metal ions.

[00191] An anti-TfR1 antibody described herein can also be conjugated (e.g., via a linker) to a second antibody (e.g., anti-beta amyloid antibody, e.g., aducanumab, bapineuzumab, gantenerumab, solanezumab, donanemab, or lecanemab; an anti-BACE1 antibody, an anti-tau antibody, an anti-alpha synuclein antibody, an anti-TDP-43 antibody, an anti-LINGO-1 antibody, an anti-LINGO-2 antibody, an anti-LINGO-3 antibody, an anti-LINGO-4 antibody, an anti-TREM2 antibody, an anti-C9orf72 dipeptide repeat poly-GA antibody, anti-CD20 antibody, anti-CD40 antibody, anti-CD40L antibody, anti-VLA4 antibody, or anti-MerTK antibody) to form an antibody heteroconjugate.

[00192] In some embodiments, the anti-TfR1 antibody of the present disclosure can be conjugated to an agent, e.g., a molecule or drug, such as an antibody, a protein (e.g., progranulin), a peptide, an enzyme (e.g., glucocerebrosidase), a nucleic acid, e.g., an antisense oligonucleotide, a short interfering RNA (siRNA), an RNA such as messenger RNA (mRNA), microRNA (miRNA), guide RNA (gRNA), a phosphoroamidate morpholino oligomer or an aptamer, etc. In some embodiments, the anti-TfR1 antibody is conjugated to a particle, e.g., lipid particle or nanoparticle, which can contain a therapeutic agent such as one described herein. In some embodiments, the anti-TfR1 antibody is conjugated to a viral particle, e.g., a viral particle comprising a therapeutic nucleic acid and/or protein, e.g., a viral particle for gene therapy (e.g., an adeno-associated virus or a lentivirus). The anti-TfR1 antibody may be linked to the agent, e.g., drug, by a linker. In some embodiments, the anti-TfR1 antibody is conjugated to a small molecule such as a cytotoxic agent (e.g., maitansine). In some embodiments, the anti-TfR1 antibody is conjugated to an anti-inflammatory agent (e.g., a glucocorticoid). In some embodiments, the anti-TfR1 antibody is conjugated to a half-life extension moiety (e.g., polyethylene glycol). Methods of preparing antibody-nucleic acid conjugates, such as the conjugates contemplated in this disclosure are well-known in the art. See, e.g., US Patent

Application Publication No. US20190240346, and US Patent Nos. US10881743 and US10550188, and International Patent Application Publication No. WO1991004753, the disclosures of which are incorporated by reference herein in their entirety.

[00193] In some embodiments, the conjugate is a fusion protein. Fusion proteins comprising an anti-TfR1 antibody described herein can be made using any suitable method known in the art. Such a fusion protein can include a fusion of an anti-TfR1 antibody of the disclosure (including bispecific, multispecific, or multivalent anti-TfR1 antibodies) with a therapeutic polypeptide or antibody (e.g., anti-beta amyloid antibody, e.g., aducanumab, bapineuzumab, gantenerumab, solanezumab, donanemab, or lecanemab; an anti-BACE1 antibody, an anti-tau antibody, an anti-alpha synuclein antibody, an anti-TDP-43 antibody, an anti-LINGO-1 antibody, an anti-LINGO-2 antibody, an anti-LINGO-3 antibody, an anti-LINGO-4 antibody, an anti-TREM2 antibody, an anti-C9orf72 dipeptide repeat poly-GA antibody, anti-CD20 antibody, anti-CD40 antibody, anti-CD40L antibody, anti-VLA4 antibody, or anti-MerTK antibody). In one instance, an anti-TfR1 antibody described herein is conjugated via a fusion protein to an anti-beta amyloid antibody (e.g., aducanumab). In one instance, an anti-TfR1 antibody described herein is conjugated via a fusion protein to rituximab. In one instance, an anti-TfR1 antibody described herein is conjugated via a fusion protein to an enzyme (e.g., iduronate 2-sulfatase, glucocerebrosidase, alpha-L-iduronidase, or sulfamidase). In one instance, avidin can be added to the C-terminus of the heavy chain to produce a fusion protein as described in Candelaria PV et al. Front Immunol. 2021;12:607692. The fusion protein may be further conjugated or complexed to a second molecule or drug, such as a biotinylated drug, as described in Daniels TR, et al. Biochim Biophys Acta. 2012;1820(3):291-317.

[00194] In some embodiments, complexes comprising an anti-TfR1 antibody described herein can be made using any suitable method known in the art. In some embodiments, the components of the complex are linked by non-covalent interactions. Such compounds comprise an anti-TfR1 antibody complexed with another agent, e.g., therapeutic agent, or complexed with a lipid or nanoparticle which has a therapeutic polypeptide or protein.

TISSUE TARGETING AND USE OF ANTI-TFR1 ANTIBODIES

[00195] In some embodiments, an anti-TfR1 antibody described can be used to target brain tissue and transport an agent across the blood brain barrier for the treatment of a

neurological disorder. Exemplary neurological disorders include Alzheimer's disease, Parkinson's disease, frontotemporal dementia, ALS, Huntington's disease, multiple sclerosis, spinal muscular atrophy, muscular dystrophy, spinal cord injury, stroke, ophthalmological conditions, acute or chronic optic neuritis, psychiatric disorders, Tourette's disease brain injury, brain tumors, and epilepsy.

[00196] Exemplary therapeutic agents for the treatment of Alzheimer's disease include caprylic triglyceride, anti-tau antibody, anti-beta amyloid antibody, anti-DKK1 antibody, APOE antagonist antibody, donepezil, quinidine, a serotonin 6 receptor antagonist, a beta-secretase inhibitor, a RAGE antagonist, a BACE inhibitor, an amyloid beta-protein inhibitor, a phosphodiesterase 9A inhibitor, bisnorcymserine, bryostatin-1, an alpha-7 potentiator, a purinoceptor P2Y6 agonist, a tau protein aggregation/TDP-43 aggregation inhibitor, N3pG-Aβ mAb, an mGlu2 agonist, quinazolinone, a mitochondrial protein stimulant, an amyloid precursor protein secretase inhibitor, a 5HT6 antagonist, R-phenserine, an amyloid beta/tau protein inhibitor, a MAO-B inhibitor, an Lp-PLA2 inhibitor, a 5-HT6 receptor antagonist, a BET protein inhibitor, an anti-protofibrillar AB mAb, nomethiazole, a histamine H3 receptor antagonist, a PPAR-delta/gamma agonist, abeotaxane, and a p38 mitogen-activated protein kinase inhibitor.

[00197] Exemplary therapeutic agents for the treatment of ALS include an anti-SOD1 antibody, anti-DR6 antibody, anti-DPR antibody, dextramipexole, arimocloamal, GM6, ibudilast, a macrophage modulator, a NOGO-A inhibitor, and a troponin complex stimulant.

[00198] Exemplary therapeutic agents for the treatment of brain injury include apomorphine, a cytokine inhibitor/neuropeptide receptor modulator, and a progesterone receptor agonist.

[00199] Exemplary therapeutic agents for the treatment of brain tumors include an IDH1 inhibitor, doxorubicin, paclitaxel, an anti-EGFRvIII antibody-drug conjugate, bevacizumab, a FGF-R kinase inhibitor, a PI3K inhibitor, cabozantinib, iodine I 131 derlotuximab biotin, a PDGFR inhibitor, carboxyamidotriazole orotate, a non-neurotoxic derivative of penclomidine, golvatinib, dexamethasone, a TGF-beta 1 kinase inhibitor, afatinib, an IDO inhibitor, cabazitaxel, a Src kinase/pre-tubulin inhibitor, a SMO protein inhibitor, an endothelin A/B receptor antagonist, a proteasome inhibitor, a T-type calcium channel antagonist, a thapsigargin analogue, irinotecan, nivolumab, a CSF-1R inhibitor, pelareorep, an EGFR antagonist, an exportin-1 protein inhibitor/nuclear protein inhibitor, a BIRC5 protein inhibitor, evofosfamide, abeotaxane, ENG protein

inhibitor, trans-sodium crocetinate, an N7-alkylating agent, a targeted anti-angiogenic agent, and veliparib.

[00200] Exemplary therapeutic agents for the treatment of epilepsy include everolimus, eslicarbazepine acetate, alprazolam, brivaracetam, carbamazepine, cannabidiol, a 4-aminobutyrate transaminase inhibitor, perampanel, a GABA-A receptor agonist, synthetic huperzine, pregabalin, clobazam, diazepam, a GABA_A synaptic and extra-synaptic receptor modulator, topiramate IV, lacosamide, and a serotonin receptor agonist.

[00201] Exemplary therapeutic agents for the treatment of genetic disorders (e.g., Friedrich's ataxia, late infantile neuronal ceroid, spinal and bulbar muscular atrophy, ataxia telangiectasia, pantothenate kinase-associated neurodegeneration, spinal muscular atrophy, familial amyloid polyneuropathy, Rett syndrome, Leigh syndrome, Wilson's disease) include a NF/E2 related factor 2 stimulant, interferon gamma-1b, rhTPP1 enzyme replacement therapy, vatiquinone, deferiprone, nusinersen, omaveloxolone, ISIS-TTRRX, a serotonin 1A receptor agonist, cytokine inhibitors/neuropeptide receptor modulator, an siRNA inhibitor targeting TTR, phosphopantothenate replacement, DcpS inhibitor, cysteamine bitartrate, indolepropionic acid, a transthyretin dissociation inhibitor, and bis-choline tetrathiomolybdate.

[00202] Exemplary therapeutic agents for the treatment of headache include an anti-CGRP mAb, a CGRP receptor antagonist mAb, sumatriptan, dextromethorphan/quinidine, onabotulinumtoxinA, a serotonin-1F receptor agonist, a nNOS inhibitor/5HT, dihydroergotamine, cyclobenzaprine, and aspirin/sumatriptan combination.

[00203] Exemplary therapeutic agents for the treatment of Huntington's disease include laquinimod, a PDE10 inhibitor, pridopidine, cysteamine bitartrate, and a VMAT2 inhibitor.

[00204] Exemplary therapeutic agents for the treatment of multiple sclerosis include natalizumab, monomethyl fumarate prodrug, anti-LINGO-1 antibody, a Nck protein modulator, a S1PR-1/5 receptor agonist, fingolimod, an anti-CD52 mAb, idebenone, a PPAR-gamma agonist/modulator, laquinimod, a tyrosine kinase inhibitor, an anti-CD19 mAb, ibudilast, guanabenz, an anti-CD20 mAb (e.g., rituximab, ocrelizumab, ofatumumab, or ublituximab), interferon beta-1b, an IL-7 receptor inhibitor, a S1P1 receptor agonist, a myelin protein stimulant, estriol succinate, imilecleucel-T, an anti-VLA 2 mAb, a BAFF-R modulator, a CD100 antigen inhibitor, an anti-DR6 antibody, and an NF-kappa B inhibitor.

- [00205]** Exemplary therapeutic agents for the treatment of muscular dystrophy include a myostatin inhibitor, drisapersen, cteplirsen, halofuginone, idebenone, ISIS-DMPKR_X, a steroid receptor agonist, a GAPDH inhibitor, a genetic transcription inhibitor, tadalafil, ataluren, and a glucocorticoid receptor agonist.
- 5 **[00206]** Exemplary therapeutic agents for the treatment of pain include a neublastin, P2X₃ purinoreceptor antagonist, a SNARE protein antagonist, oxycodone-naltrexone core (abuse resistant), amitriptyline/ketamine, rintatolimod, a cannabinoid receptor CB₂ agonist, a non-erythropoietic peptide, a PPAR-gamma agonist, a glycogen phosphorylase inhibitor, a NMDA receptor antagonist, zoledronic acid, an early growth response protein 1 inhibitor, a (histamine-3
- 10 receptor antagonist, buprenorphine, a cytokine inhibitor, cebranopadol, celecoxib, an arachidonic acid analog, a synthetic capsaicin, a Nav1.7 sodium channel inhibitor, an opioid kappa receptor agonist, duloxetine, a nerve growth factor stimulant, dexmedetomidine, a voltage-gated sodium channel inhibitor, bupivacaine, an angiotensin type 2 receptor antagonist, a nerve growth factor inhibitor, a p38 inhibitor, rapastinel, levorphanol, a CGRP mAb, pregabalin, an mGlu2/3
- 15 receptor agonist, a CACNA2D1 protein modulator, a bone resorption factor inhibitor, neublastin, a mu-opioid analgesic, an nNOS inhibitor, O-desmethyldiamadol, palmitoylethanolamide, a GABA A agonist, a TRPV-1 receptor agonist, nabiximols, a cyclo-oxygenase 2 inhibitor, a nerve growth factor modulator, cyclobenzaprine, flurbiprofen, a fatty acid amide hydrolase inhibitor, and ibuprofen/phosphatidylcholine.
- 20 **[00207]** Exemplary therapeutic agents for the treatment of Parkinson's disease include amantadine, apomorphine, an alpha7 nicotine acetylcholine receptor partial agonist, an anti-alpha-synuclein antibody, alpha-synuclein inhibitor, levodopa, a D1 potentiator, dipraglurant, a serotonin 1A/1B partial agonist, fipamezole, GM6, a retinoid X receptor agonist, istradefylline, rotigotine, pramipexole/rasagiline, R-phenserine, a serotonin 2A/6 receptor antagonist, an
- 25 adenosine A_{2A} receptor antagonist, safinamide, and a dopamine receptor agonist.
- [00208]** Exemplary therapeutic agents for the treatment of spasticity include baclofen, onabotulinumtoxinA, abobotulinumtoxinA, arbaclofen, nabiximols, and incobotulinumtoxinA.
- [00209]** Exemplary therapeutic agents for the treatment of spinal cord injury include an anti-Lingo-1 antibody, anti-NgR1 antibody, neublastin, a nervous system modulator, a Rho GTP-
- 30 binding protein-inhibitor, and fibroblast growth factor receptor.

[00210] Exemplary therapeutic agents for the treatment of stroke include natalizumab, recombinant mutant form of human wild-type activated protein C, ticagrelor, dalfampridine, aspirin, nimodipine microparticles, GM6, a PARP inhibitor, a PDZ domain inhibitor, a beta amyloid inhibitor, dabigatran, and sodium nitrite.

- 5 **[00211]** Exemplary therapeutic agents for the treatment of Tourette's Syndrome include a histamine-3 receptor antagonist, a 4-aminobutyrate transaminase inhibitor, abobotulinumtoxinA, ecopipam, a VMAT2 inhibitor, acamprostate, and vigabatrin.

- [00212]** Other exemplary therapeutic agents for the treatment of other neurological disorders include a myostatin inhibitor, NF/E2 related factor 2 stimulant, anti-tau antibody, a
 10 myeloperoxidase inhibitor, a mitochondrial permeability transition pore inhibitor, belimumab, type II-B activin receptor modulator mAb, a C1 esterase inhibitor, ferric carboxymaltose, amifampridine, fingolimod, a monoamine oxidase B inhibitor, a neurotransmitter modulator, a dopamine receptor agonist, an anti-CD19 mAb, a VMAT2 inhibitor, a CD20 mAb, thymosin beta-4, an anti-IL-6 receptor mAb, eculizumab, an AMPA receptor modulator, a steroid
 15 hydroxylase inhibitor, pyridoxal phosphate, abeotaxane, aceneuramic acid, and sodium oxybate.

- [00213]** In some embodiments, the conjugate is a fusion polypeptide comprising an anti-TfR1 antibody described herein and a whole antibody or antibody fragment (the therapeutic agent). In certain embodiments, the whole antibody or antibody fragment is an anti-beta amyloid antibody, e.g., aducanumab, bapineuzumab, gantenerumab, solanezumab, donanemab, or
 20 lecanemab, an anti-BACE1 antibody, an anti-tau antibody, an anti-alpha synuclein antibody, an anti-TDP-43 antibody, an anti-LINGO-1 antibody, an anti-LINGO-2 antibody, an anti-LINGO-3 antibody, an anti-LINGO-4 antibody, an anti-TREM2 antibody, an anti-C9orf72 dipeptide repeat poly-GA antibody (i.e., antibody capable of binding a dipeptide repeat (DPR) of poly-glycine–alanine (GA) having at least 6 repeats (GA)₆ as translated from the chromosome 9 open reading
 25 frame 72 (C9orf72) gene, an anti-CD20 antibody, an anti-CD40 antibody, an anti-CD40L antibody, an anti-VLA4 antibody, an anti-MerTK antibody, an anti-TWEAK antibody, or an anti-TWEAK-R antibody.

PHARMACEUTICAL COMPOSITIONS

[00214] The present disclosure provides compositions comprising an anti-TfR1 antibody described herein. The present disclosure also provides pharmaceutical compositions comprising an anti-TfR1 antibody described herein and a pharmaceutically acceptable vehicle.

- 5 [00215] Formulations are prepared for storage and/or use by combining an anti-TfR1 antibody of the present disclosure with a pharmaceutically acceptable vehicle (e.g., a carrier or excipient). Those of skill in the art generally consider pharmaceutically acceptable carriers, excipients, and/or stabilizers to be inactive ingredients of a formulation or pharmaceutical composition.
- 10 [00216] Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl
- 15 paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (e.g., less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrans; chelating agents such as EDTA;
- 20 sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes such as Zn-protein complexes; and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG). (*Remington: The Science and Practice of Pharmacy*, 22nd Edition, 2012, Pharmaceutical Press, London.). In some embodiments, the formulation is in the form of an aqueous solution. In some embodiments, the formulation is lyophilized or in an alternative
- 25 dried form.

- [00217] The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include
- 30 corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and diluents (e.g., water). These can be used to form a solid preformulation

composition containing a homogeneous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. The solid preformulation composition is then subdivided into unit dosage forms of a type described above. The tablets, pills, etc. of the formulation or composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[00218] The binding agents of the present disclosure may be formulated in any suitable form for delivery to a target cell/tissue. In some embodiments, an anti-TfR1 antibody can be formulated as a liposome, microparticle, microcapsule, albumin microsphere, microemulsion, nano-particle, nanocapsule, or macroemulsion. In some embodiments, the pharmaceutical formulation includes an anti-TfR1 antibody of the present disclosure complexed with liposomes. Methods to produce liposomes are known to those of skill in the art. For example, some liposomes can be generated by reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE).

[00219] In some embodiments, an anti-TfR1 antibody is formulated as a sustained-release preparation. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing an agent, where the matrices are in the form of shaped articles (e.g., films or microcapsules). Sustained-release matrices include but are not limited to polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(vinyl alcohol), polylactides, copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(–)-3-hydroxybutyric acid.

[00220] The pharmaceutical compositions or formulations of the present disclosure can be administered in any number of ways for either local or systemic treatment. In some embodiments, administration is topical by epidermal or transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. In some embodiments,

administration is pulmonary by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, and intranasal. In some embodiments, administration is oral. In some embodiments, administration is parenteral including intravenous, intraarterial, intratumoral, subcutaneous, intraperitoneal, intramuscular (e.g., injection or infusion), or intracranial (e.g., intrathecal or intraventricular). In some embodiments, administration is by intravenous injection or intravenous infusion. In some embodiments, administration is by intramuscular injection. In some embodiments, administration is intrathecal (e.g., intrathecal administration via injection directly into the intrathecal space or injection into a port for intrathecal delivery). In some embodiments, administration subcutaneous.

10 [00221] Various delivery systems are known and can be used to administer an anti-TfR1 antibody described herein. In some embodiments, an anti-TfR1 antibody or a composition described herein is delivered in a controlled release or sustained release system. In some embodiments, a pump is used to achieve controlled or sustained release. In some embodiments, polymeric materials are used to achieve controlled or sustained release of the anti-TfR1 antibody
15 herein. Examples of polymers used in sustained release formulations include, but are not limited to, poly 2-hydroxy ethyl methacrylate, polymethyl methacrylate, polyacrylic acid, polyethylene-co-vinyl acetate, polymethacrylic acid, polyglycolides (PLG), polyanhydrides, poly N-vinyl pyrrolidone, polyvinyl alcohol (PVA), polyacrylamide, polyethylene glycol (PEG), polylactides (PLA), polylactide-co-glycolides (PLGA), and polyorthoesters. Any polymer used in a sustained
20 release formulation should be inert, free of leachable impurities, stable on storage, sterile, and biodegradable.

[00222] Additional delivery systems can be used to administer an anti-TfR1 antibody described herein including, but not limited to, injectable drug delivery devices and osmotic pumps. Injectable drug delivery devices include, for example, hand-held devices (e.g.,
25 autoinjectors) or wearable devices. Different types of osmotic pump systems may include single compartment systems, dual compartment systems, and multiple compartment systems.

EXEMPLARY EMBODIMENTS

30 [00223] Exemplary embodiments as described below are also within the scope of the present disclosure:

Embodiment 1. An antibody that binds to human transferrin receptor, comprising a heavy chain variable region (VH) comprising VH complementarity determining region (CDR)1, VH CDR2, and VH CDR3, and a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3, wherein:

the VH CDR1 comprises the amino acid sequence GFTFSSYX₁MN (SEQ ID NO:18) or the amino acid sequence SYX₁MN (SEQ ID NO:26), wherein X₁ is S or A;

the VH CDR2 comprises the amino acid sequence SISX₂SSSX₃IYYADSVKG (SEQ ID NO:19), wherein X₂ is S or A, and wherein X₃ is Y or S; and

the VH CDR3 comprises the amino acid sequence KX₄X₅X₆GDFDY (SEQ ID NO:20), wherein X₄ is Y or S, wherein X₅ is R or S, and wherein X₆ is A or Y;

the VL CDR1 comprises the amino acid sequence RASQSVSSX₇X₈LA (SEQ ID NO:21), wherein X₇ is S or N, and wherein X₈ is Y or N;

the VL CDR2 comprises the amino acid sequence GASX₉RAT (SEQ ID NO:22),

wherein X₉ is N or S; and

the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8).

Embodiment 2. The antibody of embodiment 1, wherein at least one of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1, and any of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3 that are not selected from the mutant CDRs depicted in Table 1 are selected from the parental CDRs depicted in Table 1.

Embodiment 3. The antibody of embodiment 1, wherein one of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3 is selected from the mutant CDRs depicted in Table 1 and five of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1.

Embodiment 4. The antibody of embodiment 1, wherein two of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in

Table 1 and four of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1.

Embodiment 5. The antibody of embodiment 1, wherein three of VH CDR1, VH CDR2, VH
5 CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and three of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1.

Embodiment 6. The antibody of embodiment 1, wherein four of VH CDR1, VH CDR2, VH
10 CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and two of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1.

Embodiment 7. The antibody of embodiment 1, wherein five of VH CDR1, VH CDR2, VH
15 CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and one of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3 is selected from the parental CDRs depicted in Table 1.

Embodiment 8. The antibody of embodiment 1, wherein:
20 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
25 the amino acid sequence QQSSSPPT (SEQ ID NO:8); or

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH
CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH
CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
30 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQSSSPPT (SEQ ID NO:8).

Embodiment 9. The antibody of embodiment 1, wherein:

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the
5 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S
conservative substitution;

10 the VH CDR1 comprises the amino acid sequence GFTFSSYAMN (SEQ ID NO:9); the
VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
15 the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
20 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
25 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S
conservative substitution;

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
30 VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1

comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
 5 VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:11); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

10 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KSRAGDFDY (SEQ ID NO:12); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
 15 the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
 20 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRYGDFDY (SEQ ID NO:14); the VL CDR1
 25 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSIYYADSVKG (SEQ ID NO:25); the
 30 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
5 VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
10 VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
15 VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNLA (SEQ ID NO:16); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
25 comprises the amino acid sequence GASSRAT (SEQ ID NO:17); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
30 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises

the amino acid sequence QQQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence SYAMN (SEQ ID NO:24); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:11); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KSRAGDFDY (SEQ ID NO:12); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRYGDFDY (SEQ ID NO:14); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSSYIYYADSVKG (SEQ ID NO:25); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNLA (SEQ ID NO:16); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8); or

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASSRAT (SEQ ID NO:17); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8).

Embodiment 10. The antibody of any one of the preceding embodiments, wherein:

- (i) the VH is at least 80%, 85%, 90%, 95%, 99%, or 100% identical to any one of SEQ ID NOs:100-108; and
- (ii) the VL is at least 80%, 85%, 90%, 95%, 99%, or 100% identical to any one of SEQ ID NOs:200-204.

Embodiment 11. The antibody of embodiment 1, wherein the VH comprises the amino acid sequence of any one of SEQ ID NOs:100-108 and the VL comprises the amino acid sequence of any one of SEQ ID NOs:200-204.

Embodiment 12. The antibody of any one of the preceding embodiments, wherein the antibody is (a) monovalent and has a monovalent affinity (K_D) for hTfR1 of > 10 nM or is bivalent and has a monovalent affinity (K_D) for hTfR1 of >100 nM and/or (b) has an off rate (k_d) of $\geq 0.01/s$.

5 Embodiment 13. The antibody of embodiment 1, wherein:

the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:200.

Embodiment 14. The antibody of embodiment 1, wherein:

10 the VH comprises the amino acid sequence of SEQ ID NO:101 and the VL comprises the amino acid sequence of SEQ ID NO:200;

the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO: 200;

15 the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO:201;

the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO:203;

the VH comprises the amino acid sequence of SEQ ID NO:103 and the VL comprises the amino acid sequence of SEQ ID NO:200;

20 the VH comprises the amino acid sequence of SEQ ID NO:104 and the VL comprises the amino acid sequence of SEQ ID NO:200;

the VH comprises the amino acid sequence of SEQ ID NO:105 and the VL comprises the amino acid sequence of SEQ ID NO:200;

25 the VH comprises the amino acid sequence of SEQ ID NO:106 and the VL comprises the amino acid sequence of SEQ ID NO:200;

the VH comprises the amino acid sequence of SEQ ID NO:107 and the VL comprises the amino acid sequence of SEQ ID NO:200;

the VH comprises the amino acid sequence of SEQ ID NO:108 and the VL comprises the amino acid sequence of SEQ ID NO:200;

30 the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:201;

the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:202;

the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:203; or

- 5 the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:204.

Embodiment 15. The antibody of any one of embodiments 1 to 14, which is a multispecific antibody, bispecific antibody, single chain antibody, an Fab fragment, an F(ab')₂ fragment, an
10 Fab' fragment, an Fsc fragment, an Fv fragment, an scFv, an sc(Fv)₂, or a diabody.

Embodiment 16. The antibody of any one of embodiments 1 to 14, comprising a constant heavy chain (CH) domain and a constant light chain (CL) domain.

- 15 Embodiment 17. The antibody of embodiment 1, wherein the antibody comprises:
- (a) a heavy chain comprising the amino acid sequence set forth in 400, and a light chain comprising the amino acid sequence set forth in 401;
 - (b) the amino acid sequences set forth in SEQ ID NOs: 401 and 402;
 - (c) the amino acid sequences set forth in SEQ ID NOs: 401, 404, and 406;
 - 20 (d) the amino acid sequences set forth in SEQ ID NOs:400 and 401;
 - (e) the amino acid sequences set forth in SEQ ID NOs: 401, 405, and 406; or
 - (f) the amino acid sequences set forth in SEQ ID NOs: 401 and 403.

Embodiment 18. A nucleic acid or nucleic acids encoding the antibody of any one of
25 embodiments 1 to 17.

Embodiment 19. An expression vector or expression vectors comprising the nucleic acid or nucleic acids of embodiment 18 operably linked to a promoter.

30 Embodiment 20. An isolated cell comprising the nucleic acid or nucleic acids of embodiment 18 or the expression vector or expression vectors of embodiment 19.

Embodiment 21. An isolated cell comprising a first expression vector comprising a first nucleic acid encoding a first polypeptide comprising the VH of the antibody of any one of embodiments 1 to 17 operably linked to a promoter, and a second expression vector comprising a second
5 nucleic acid encoding a second polypeptide comprising the VL of the antibody of any one of embodiments 1 to 17 operably linked to a promoter.

Embodiment 22. A method of making the antibody of any one of embodiments 1 to 17, comprising culturing the cell of embodiment 20 or 21 and isolating the antibody.

10

Embodiment 23. A pharmaceutical composition comprising the antibody of any one of embodiments 1 to 17 and a pharmaceutically acceptable carrier.

Embodiment 24. A conjugate comprising the antibody of any one of embodiments 1 to 17 and
15 an agent.

Embodiment 25. The conjugate of embodiment 24, wherein the agent is an antibody, protein, or peptide.

20 Embodiment 26. The conjugate of embodiment 24, wherein the agent is an anti-beta amyloid antibody.

Embodiment 27. The conjugate of embodiment 26, wherein anti-beta amyloid antibody is aducanumab, bapineuzumab, gantenerumab, solanezumab, donanemab, or lecanemab.

25

Embodiment 28. The conjugate of embodiment 24, wherein the agent is an anti-tau antibody, an anti-alpha synuclein antibody, an anti-TDP-43 antibody, an anti-LINGO-1 antibody, an anti-LINGO-2 antibody, an anti-LINGO-3 antibody, an anti-LINGO-4 antibody, an anti-TREM2 antibody, or an anti-C9orf72 dipeptide repeat poly-GA antibody.

30

Embodiment 29. The conjugate of embodiment 24, wherein the agent is protein.

Embodiment 30. The conjugate of embodiment 29, wherein the protein is progranulin.

Embodiment 31. The conjugate of embodiment 24, wherein the agent is an enzyme.

5

Embodiment 32. The conjugate of embodiment 31, wherein the enzyme is glucocerebrosidase.

Embodiment 33. The conjugate of any one of embodiments 24 to 32, wherein the conjugate is a recombinant fusion protein comprising the antibody and the agent.

10

Embodiment 34. The conjugate of embodiment 24, wherein the agent is a nucleic acid.

Embodiment 35. The conjugate of embodiment 34, wherein the nucleic acid is an mRNA, a siRNA, an antisense oligonucleotide, microRNA (miRNA), guide RNA (gRNA), or a phosphoroamidate morpholino oligomer (PMO).

15

Embodiment 36. The conjugate of embodiment 34 or 35, wherein the nucleic acid is linked to the antibody via a linker.

20 Embodiment 37. The conjugate of embodiment 24, wherein the agent is a nanoparticle, liposome, or viral vector.

Embodiment 38. A method of transporting an agent across the blood brain barrier via transcytosis, the method comprising administering to a human subject the conjugate of any one of embodiments 24 to 37.

25

Embodiment 39. A method of delivering an agent *in vivo*, the method comprising administering to a human subject the conjugate of any one of embodiments 24 to 37.

30 Embodiment 40. The method of embodiment 39, wherein the human subject has a neurological disorder and the method delivers the agent to brain tissue.

Embodiment 41. The method of embodiment 39, wherein the neurological disorder is Alzheimer's disease, Parkinson's disease, frontotemporal dementia, ALS, Huntington's disease, multiple sclerosis, spinal muscular atrophy, muscular dystrophy, spinal cord injury, stroke, an
5 ophthalmological condition, acute or chronic optic neuritis, a psychiatric disorder, Tourette's disease brain injury, a brain tumor, or epilepsy.

Embodiment 42. A method of treating Alzheimer's disease in a human subject in need thereof, comprising administering to the human subject a therapeutically effective amount of the
10 conjugate of embodiment 26 or 27.

[00224] The following examples are provided to better illustrate the claimed invention and are not to be interpreted as limiting the scope of the invention. To the extent that specific materials are mentioned, it is merely for purposes of illustration and is not intended to limit the
15 invention. One skilled in the art can develop equivalent means or reactants without the exercise of inventive capacity and without departing from the scope of the invention.

EXAMPLES

EXAMPLE 1: ANTIBODY GENERATION

20 [00225] Adimab expression libraries were screened in accordance with the methods disclosed in US Patent Publications 20100056386 and 20090181855. After iterative rounds of selective pressure towards human TfR1 ectodomain (ECD), followed by selective pressure towards cynomolgus monkey TfR1 ECD, followed by selected pressure towards human TfR1 ECD pre-blocked with recombinant human holo-transferrin (Sigma; T0665). Colonies were
25 subsequently sequenced to identify unique clones, using techniques known in the art. Following this campaign, 768 antibodies were expressed from yeast and purified on protein A resin from yeast. Yeast-expressed antibodies were tested for binding to both human and cyno TfR ECD recombinant protein using Bio-Layer Interferometry (BLI). Antibody binding was similarly performed against human TfR after pre-blocking of human TfR ECD with recombinant human
30 holo-transferrin. BLI was performed on an Octet RED384 and Octet HTX instruments manufactured by ForteBio according to standard procedures. Yeast-expressed antibodies from

the BLI triage were then tested for binding to BAF3 cells over-expressing full length human TfR (huTfR-CHO) or cynomolgus monkey TfR (cyTfR-CHO). Cells were incubated with antibodies at a single 200nM concentrations for 2 hours on ice, washed twice with isotonic buffer, then incubated with a fluorescently labeled anti-IgG secondary antibody (Jackson; 109-116-097, fixed in 1% PFA and analyzed on a flow cytometer.

[00226] Antibodies were expressed as aglycosylated hIgG1 by transient transfection of suspension CHO-S cells in serum-free medium. Conditioned supernatant was collected by centrifugation and filtration. Proteins were purified by loading supernatants on Protein A Sepharose FF, eluting with 25 mM sodium phosphate, 100 mM NaCl, pH 2.8, then neutralizing with 1:60 (v:v) 500 mM sodium phosphate pH 8.6. Further purification was performed by passing neutralized eluates over TMAE anion exchange resin equilibrated in PBS pH 7.0. Aggregate content was assessed and, if >5% of total protein, antibodies were further purified by size exclusion chromatography (superdex 200 in PBS).

[00227] Antibodies were evaluated for TfR binding, Tf blocking, and transcytosis properties. For cell binding studies, bivalent antibodies were bound to CHO cells expressing full length human TfR1 (huTfR-CHO) or cynomolgus monkey TfR1 (cyTfR-CHO), without endogenous hamster TfR1 (TfR KO CHO background). Cells were incubated with antibodies at concentrations up to 1000 nM (4-fold 7-pt dilution series) for 1-2 hours on ice, then washed three times with isotonic buffer, incubated with fluorescent secondary reagent (PE conjugated) that binds human IgG, washed again, fixed in 1% paraformaldehyde and analyzed on a flow cytometer. Mean fluorescence intensity (MFI) of the PE fluorophore was calculated and represents binding of antibody to cells. Non-specific binding was assessed by binding of antibodies at 1000 nM to TfR KO CHO cells. Transferrin competition binding was assessed by comparing titration binding of the antibodies to huTfR-CHO in the absence and presence of 1000nM human holo-transferrin (Sigma T0665). The results for ANTIBODY X, Antibody 1, and Antibody 2 are provided in **Table 7**.

[00228] For the transcytosis assay, Caco2 cells were seeded at 25,000/well in Corning 0.4 micron transwell inserts and cultured for 21 days in complete medium (DMEM, 10% FBS, 1mM sodium pyruvate, 2mM Glutamine, 1% NEAA, and 1% Pen/Strep). Trans-epithelial electrical resistance (TEER) values were in the range 1000-2000 Ohm.cm². Antibodies were added to the top well at 100nM and two days later, samples were collected from the bottom well, then

analyzed for hIgG concentration using commonly available anti-human IgG reagents in a mesoscale discovery assay system, with results extrapolated from a titrated standard curve of the same test article. Results were reported as a ratio of the bottom well concentration for the test anti-TfR antibody over that measured for a non-targeted hIgG control antibody in a separate transwell. The results for ANTIBODY X, Antibody 1, and Antibody 2 are provided in **Table 7**.

Table 7.

Anti-TfR	huTfR-CHO EC ₅₀ (nM)	cyTfR-CHO EC ₅₀ (nM)	TfR KO CHO MFI at 1 uM	Tf block	Caco2 Transcytosis (fold/control)
ANTIBODY X	1.8	1.5	7	No	7
Antibody 1	0.3	~0.6	47	Yes	6
Antibody 2	12	5.4	3	Yes	5

[00229] ANTIBODY X was selected for a combination of well-matched binding to human and cynomolgus monkey TfR1, low non-specific cell binding, no competition with transferrin, and positive transcytosis in Caco2 cells (**Table 7**).

[00230] The amino acid sequence of the variable regions of ANTIBODY X are provided below.

[00231] ANTIBODY X VH:

EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSYIYYADSVKGRFTISR
DNAKNSLYLQMNSLRAEDTAVYYCARKYRAGDFDYWGQGTLLVTVSS (SEQ ID NO:100)

[00232] ANTIBODY X VL:

EIVMTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASNRAATGIPDRFSGSGSGTDFT
LTISRLEPEDFAVYYCQQSSSPPTFGGGTKVEIK (SEQ ID NO:200)

EXAMPLE 2: CHARACTERIZATION OF ANTIBODY X

[00233] Affinity and kinetics of ANTIBODY X binding to transferrin receptor was assessed by surface plasmon resonance (SPR). Histidine tagged human or cynomolgus monkey transferrin receptor-1 ectodomain (huTfR1 ECD or cyTfR1 ECD, respectively) was captured at

30-50 pg/mm² on a SPR chip (CM5 chip in a Biacore 8K+) coated with anti-His capture reagent (Cytiva). Monovalent antibodies or Fab fragments comprising the VH and VL of ANTIBODY X (digested with papain, Roche 108014 at 10 ug per mg of antibody incubated at 37 °C for 4 hours) were injected at concentrations ranging from 4 to 4000 nM. Binding sensograms (**FIG. 1A and FIG. 1B**) were measured and analyzed with a 1:1 binding model using Biacore Insight Evaluation Software. Rapid dissociation kinetics ($k_d \sim 0.02$ /s) and modest monovalent affinity ($K_D \sim 50$ nM), as desired for functional binding, transport and release at the blood-brain barrier, were observed for ANTIBODY X.

[00234] Further investigation of binding properties of monovalent hIgG formats, where the ANTIBODY X Fab is fused to either the N-terminus or C-terminus of a human Fc fragment using knob-in-hole mutations to drive heterodimerization with an Fc chain without Fab (Fab-Fc or Fc-Fab, respectively), were performed as described above. No significant differences in affinity for huTfR1 ECD were observed between monovalent antibody formats, although binding to cyTfR1 ECD was slightly weaker for the Fc fusions than the Fab fragment (**Table 8**).

[00235] Differences in binding cell surface human TfR1 for different format variants, including monovalent and bivalent formats, were investigated by flow cytometry. CHO cells with endogenous hamster TfR knocked out and human TfR over-expressed, were incubated with antibodies (0.001-1000 nM) containing the Fab region from ANTIBODY X, including Fab-Fc and Fc-Fab bivalent fusions, and the two monovalent formats Fab-Fc and Fc-Fab, on ice for 60-90 minutes, washed three times, then incubated with fluorescently labeled anti-hIgG Fc (Jackson 109-116-098) for 60 minutes, fixed in 1% PFA, and analyzed in a flow cytometer. The monovalent antibody formats had weaker binding than the bivalent formats (**FIG. 1C, Table 8**). Differences between Fab-Fc and Fc-Fab formats were observed in flow cytometry that were not evident by SPR (**FIG. 1C, Table 8**).

[00236] To evaluate competitive binding with transferrin, binding to TfR1 ECD by SPR for antibody alone was compared to antibody with holo human transferrin at 1000nM (T0665, Sigma) pre-saturated on the captured receptor, mixed with the antibody, and included during the dissociation phase (in ABA mode in the Biacore control software). No weakening of binding affinity was observed with transferrin present, rather slightly stronger binding was detected (**Table 8**).

[00237] Cell surface transferrin competition experiments were performed by flow cytometry. Binding of ANTIBODY X Fab-Fc bivalent (0.001-1000 nM) to huTfR CHO was evaluated in the absence and presence of holo human transferrin (1000 nM). Similar to what was observed by SPR, there was no loss of antibody binding in the presence of transferrin, rather
 5 there was slightly elevated levels of antibody staining with transferrin present (**FIG. 2A**).

[00238] Transferrin displacement by ANTIBODY X Fab-Fc bivalent was characterized by incubating huTfR CHO with sub-saturating levels of AlexaFluor647-labeled human transferrin (3nM, Thermo T23366) together with anti-TfR or unlabeled holo human transferrin. Minimal transferrin displacement was observed for ANTIBODY X Fab-Fc bivalent compared to a
 10 positive blocking anti-TfR (Antibody 1) or holo human transferrin (**FIG. 2B**).

Table 8.

ANTIBODY X Protein description	Antibody molecule comprises the following polypeptide chain sequences:	huTfR ECD k_a ($M^{-1}s^{-1}$)	huTfR ECD k_d (s^{-1})	huTfR ECD K_D (nM)	huTfR CHO EC_{50} (nM)
Fab fragment	SEQ ID NO:401 SEQ ID NO:402	5.0×10^5	2.1×10^{-2}	43	
Fab-Fc monovalent	SEQ ID NO:401 SEQ ID NO:404 SEQ ID NO:406	3.8×10^5	2.1×10^{-2}	55	47
Fab-Fc monovalent + holo huTf (1uM)	SEQ ID NO:401 SEQ ID NO:404 SEQ ID NO:406	4.4×10^5	2.2×10^{-2}	49	
Fc-Fab monovalent	SEQ ID NO:401 SEQ ID NO:405 SEQ ID NO:406	3.2×10^5	1.7×10^{-2}	53	6.9
Fc-Fab monovalent + holo huTf (1uM)	SEQ ID NO:401 SEQ ID NO:405 SEQ ID NO:406	3.5×10^5	1.7×10^{-2}	48	
Fab-Fc bivalent	SEQ ID NO:400				0.20

	SEQ ID NO:401				
Fc-Fab bivalent	SEQ ID NO:401 SEQ ID NO:403				0.45
ANTIBODY X Protein description	Antibody molecule comprises the following polypeptide chain sequences:	cyTfR ECD k_a ($M^{-1}s^{-1}$)	cyTfR ECD k_d (s^{-1})	cyTfR ECD K_D (nM)	cyTfR CHO EC_{50} (nM)
Fab fragment	SEQ ID NO:401 SEQ ID NO:402	5.6×10^5	3.7×10^{-2}	67	
Fab-Fc monovalent	SEQ ID NO:401 SEQ ID NO:404 SEQ ID NO:406	2.6×10^5	3.2×10^{-2}	125	84
Fab-Fc monovalent + holo huTf (1uM)	SEQ ID NO:401 SEQ ID NO:404 SEQ ID NO:406	2.8×10^5	3.2×10^{-2}	115	
Fc-Fab monovalent	SEQ ID NO:401 SEQ ID NO:405 SEQ ID NO:406	2.4×10^5	3.0×10^{-2}	128	21
Fc-Fab monovalent + holo huTf (1uM)	SEQ ID NO:401 SEQ ID NO:405 SEQ ID NO:406	2.8×10^5	3.1×10^{-2}	111	
Fab-Fc bivalent	SEQ ID NO:400 SEQ ID NO:401				0.13
Fc-Fab bivalent	SEQ ID NO:401 SEQ ID NO:403				0.59

EXAMPLE 3: ANTIBODY X PARATOPE MAPPING

[00239] To identify the molecular basis for antigen recognition of ANTIBODY X, a cryo-EM structure was generated of the ternary complex of ANTIBODY X Fab fragment, huTfR1
5 ECD, and holo human transferrin (Sigma T0665) to a resolution of 3.6 Å. An atomic model of the complex was obtained by fitting a homology model of ANTIBODY X Fab and available crystal structures of TfR1 and Tf into the cryo-EM density map (**FIG. 3A**). The final model

revealed that ANTIBODY X binds to a conformational epitope spanning the apical and protease-like domains of TfR1. There are 17 residues from TfR1 and 19 residues from the ANTIBODY X Fab that are within 4 Å of the binding partner. The discontinuous epitope is formed by residues Q285, T286, K287, P289, E343, D352, C353, P354, S355, K358, D360, S361, R364 from the apical domain and S492, D560, T561, and R579 from the protease-like domain of hu TfR1. The antigen-binding site (based on the atomic distances in the cryo-EM model) of ANTIBODY X is formed by all six CDRs (VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and VL CDR3) and is composed of S32, S33, S40, S60, S61, S66, Y67, K109, Y110, R111, and A112 of the heavy chain (AHO numbering of SEQ ID NO:100), and S33, S39, Y40, N69, Q109, and S110 of the light chain (AHO numbering of SEQ ID NO:200), plus additional framework contacts from Y57 and S83 of the light chain (**Table 9**).

[00240] ANTIBODY X recognizes TfR1 in humans and cynomolgus monkeys. These structural studies provide detailed insights into the binding properties of ANTIBODY X. The binding epitope in the apical and protease-like domains of TfR1 is highly conserved between human and cynomolgus monkey, with only a single conservative R364K mutation (hu>cyno TfR1) (**FIG. 3B**), consistent with the cross-reactivity of binding of ANTIBODY X from these species.

Table 9: Anti-TfR ANTIBODY X paratope residues that contact TfR1. Contact residues within 4.0 Å of TfR1 are in bold, lowercase.

CDR	Contact residue (AHO) (<4Å)	CDR sequence
VH CDR1	S32, S33, S40	GFTFssYsMN (SEQ ID NO:3)
VH CDR2	S60, S61, S66, Y67	SISssSsyIYYADSVKG (SEQ ID NO:4)
VH CDR3	K109, Y110, R111, A112	kyra GDFDY (SEQ ID NO:5)
VL CDR1	S33, S39, Y40	RASQSVSsyLA (SEQ ID NO:6)
VL CDR2	N69	GASnRAT (SEQ ID NO:7)
VL CDR3	Q109, S110	QQ qs SSPPT (SEQ ID NO:8)
LC Framework	Y57, S83	

EXAMPLE 4: CELLULAR TRAFFICKING OF ANTIBODY X

[00241] To investigate the cellular trafficking of ANTIBODY X, pH dependent binding to transferring receptor and transcytosis in model blood brain barrier (BBB) cells was characterized.

5 [00242] Since the pH drops from cell surface along the endosomal, lysosomal, and transcellular trafficking pathways, the pH-dependent binding of anti-TfR antibodies may affect the sorting into different sub-cellular compartments and may affect efficiency of transcytosis. The binding of ANTIBODY X in Fab-Fc monovalent format at 2 – 2000 nM to His-tagged hTfR1 ECD was measured at pH 7.4 and pH 5.5, by surface plasmon resonance (as described
10 above). A much lower binding response was measured at pH 5.5 as compared to pH 7.4 (**FIG. 4A**), despite similar levels of hTfR1 ECD on the SPR chip, suggesting a strong pH sensitivity of binding for ANTIBODY X.

[00243] To understand if changes in antibody format/valency can improve cellular transport, transcytosis of ANTIBODY X in multiple antibody formats was tested in Caco2 cells
15 cultured in transwells, as described above. After two days of incubation in the Caco2 transwell top-well at 100nM, ANTIBODY X formatted as Fab-Fc bivalent was detected in the bottom well at 7.6x higher levels than a non-targeted hIgG control antibody, whereas ANTIBODY X formatted as Fc-Fab bivalent, Fab-Fc monovalent and Fc-Fab monovalent each showed significantly higher transcytosis, at 15-20x higher levels than control hIgG (**FIG. 4B**). These
20 data demonstrate that each of the tested ANTIBODY X formats was active for *in vitro* cellular internalization and transcytosis and that the format of ANTIBODY X can alter transcytosis degree, rendering ANTIBODY X in various formats useful for various applications depending on, *e.g.*, the desired degree of transcytosis and the cargo attached.

25 **EXAMPLE 5: BRAIN DISTRIBUTION OF ANTIBODY X**

[00244] To test whether ANTIBODY X could penetrate the blood-brain barrier *in vivo*, human TfR knock-in mice (hTfR KI, containing the ANTIBODY X epitope) were injected intravenously with 20 mg/kg of ANTIBODY X Fab-Fc bivalent or non-targeted control hIgG, both containing the mutation N297Q to reduce Fc effector function through aglycosylation. In
30 life serum samples were collected via cheek bleeds. After 4, 24, 72, or 168 hours, mice were

anesthetized with ketamine/xylazine (100/10 mg/kg i.p.), blood samples were collected via cardiac puncture, and mice were perfused, through the left ventricle, with ice-cold PBS/Heparin (1ug/mL) at 2ml/minute for 10 minutes to clear the vasculature of blood. Brains were then removed and hemisected, with one hemisphere flash frozen in liquid nitrogen and the other hemisphere fixed in 10% neutral buffered formalin for 24 hours. Blood samples were analyzed for complete blood cell count, including reticulocytes, within 24 hours of collection (IDEXX). Additionally, serum was generated by allowing blood to clot for 15-30 minutes at room temperature, centrifugation at 2000g for 10 minutes, freezing supernatants for further analysis.

[00245] Frozen brain hemispheres were homogenized in lysis buffer (50 mM Tris pH 7.5, 150 mM NaCl, 0.25 % Na deoxycholate, 1 mM EDTA, 1 % NP40, complete Protease inhibitors) with zirconium oxide beads (ZROB05 and ZROB10) in a tissue homogenizer (NextAdvance Bullet Blender) for 10 minutes, then incubated at 4 °C, rotating for 1 hour. Lysates were then cleared of debris by centrifugation at 12,000g for 20 minutes.

[00246] Human IgG levels were analyzed in serum and brain lysate by MSD immunoassay. Samples were incubated on MSD plates (MSD, Cat# L15XB-3/L11XB-3) coated with anti-hIgG Fc capture reagent (Jackson ImmunoResearch, Cat# 709-006-098), detected with sulfo-tagged anti-hIgG (MSD, Cat# R32AJ-1), and quantified by interpolation on a standard curve generated for each test antibody.

[00247] Formalin-fixed brain hemispheres were transferred into phosphate buffered saline, paraffin embedded, separated into six 5mm coronal segments, sectioned 3-5 mm thick, proteinase-K treated for antigen retrieval, and stained for human IgG (Southern Biotech 6145-01, 2ug/ml, then Leica Refine HRP polymer and DAB chromogen).

[00248] ANTIBODY X showed much faster clearance than control IgG in serum, dropping by >10x by 3 days and >1000x by 7 days following IV administration (**FIG. 5A**), as expected for transferrin receptor-mediated disposition. Reticulocyte counts were transiently reduced to ~1% of total blood cells at 1 day for ANTIBODY X, compared to ~3% for control hIgG, but recovered to ~3% by 7 days. Brain exposure was ~10x greater for ANTIBODY X than control hIgG up to 3 days after IV administration but fell to less than control hIgG at 7 days (**FIG. 5B**). Immunohistochemistry results, staining for hIgG, showed that ANTIBODY X has enhanced biodistribution across multiple brain regions (particularly in cerebral cortex) relative to

non-targeted control hIgG (**FIG. 5C**; images from day 3). At higher magnification hIgG staining is seen on blood vessels as well as throughout the parenchyma and on pyramidal neurons in the cerebral cortex (**FIG. 5D**). Overall, ANTIBODY X shows greatly enhanced biodistribution to the CNS.

5 **[00249]** To validate the translatability of the brain uptake activity of ANTIBODY X in non-human primate, cynomolgus monkeys were administered an IV dose of 20 mg/kg of Fab-Fc bivalent ANTIBODY X or non-targeted control hIgG. After 48 hours, following similar methods as for the mouse studies described above, animals were perfused under ketamine/xylazine anesthesia and brain samples were collected from frontal cortex, hippocampus, and cerebellum from each hemisphere. Brain biopsies from one hemisphere were flash frozen, homogenized, and analyzed for total hIgG by MSD immunoassay. Brain biopsies from the second hemisphere were formalin fixed processed for hIgG immunohistochemistry.

10 **[00250]** ANTIBODY X was detected in brain biopsies at 20-25 times higher levels than control hIgG in all regions sampled (**FIG. 6A**). As was observed in hTfR KI mice, the elevated brain uptake of ANTIBODY X corresponds to increased vascular, parenchymal and neuronal hIgG staining by IHC (**FIG. 6B**). Furthermore, ANTIBODY X was detected in vesicles within pyramidal neurons of the cerebral cortex (**FIG. 6C**), which supports therapeutic applications requiring neuronal lysosomal delivery.

20 **EXAMPLE 6: DOSE-DEPENDENT BRAIN EXPOSURE OF ANTIBODY X**

25 **[00251]** Receptor-mediated transport at the BBB is expected to be a saturable process, with the capacity for delivery being dependent on expression of the receptor in brain endothelial cells. To better understand the limits of TfR1 mediated brain uptake by ANTIBODY X, a dose-dependent biodistribution study was performed in hTfR KI mice. As ANTIBODY X has higher brain uptake in monovalent format compared to bivalent format, likely due to 1:1 stoichiometry of TfR1 interaction, it was dosed as a monovalent Fab-Fc fusion (three chain molecule consisting of an aglycosylated heavy chain, light chain, and Fc fragment, utilizing knob-in-hole mutations for Fc heterodimerization) at 3, 10, 30, and 90 mg/kg molar IgG equivalent IV (i.e. 2, 6.7, 20, and 60 mg/kg Fab-Fc) and compared to a non-targeted bivalent control antibody at equivalent

doses. One day later, blood and cardiac-perfused brain samples were collected from each mouse and hIgG levels in serum and brain lysate were quantified by MSD immunoassay.

[00252] Serum exposure increased in proportion to increasing dose (**FIG. 17A**) but was lower for the monovalent ANTIBODY X than for the bivalent hIgG control, by a factor that was consistent across all dose levels (0.3-0.45 fold/control). This suggests that TfR1-mediated clearance effects were not significant at this 1-day time point. Brain exposure, however, was higher for monovalent ANTIBODY X than for the hIgG control at all dose levels (**FIG. 17B**). Monovalent ANTIBODY X also showed a non-linear dose-dependence of brain uptake, reaching ~15 nM in the brain from a 10 mg/kg molar IgG equivalent dose, but increasing to only 25-30 nM for a 90 mg/kg molar IgG equivalent dose. This modest increase in brain exposure with increasing dose above 10 mg/kg is likely due to passive biodistribution, as the control IgG showed a similar increase in brain uptake in this dose range. As such, the improvement in brain exposure, expressed as fold-over-control, is highest at the lowest dose tested, but drops for higher doses due to saturation of TfR1-mediated transport and the increase in passive brain uptake (**FIG. 17C**).

EXAMPLE 7: ANTIBODY X-ANTI-BACE1

[00253] To demonstrate anti-TfR mediated delivery of an antibody to the CNS at sufficient concentrations to achieve brain-specific pharmacologic activity, bispecific antibodies combining the ANTIBODY X and anti-Beta Secretase1 (BACE1) antibody with affinity of $K_D = 1.3$ nM for recombinant human and mouse BACE1 ECD were tested. Two versions of this bispecific were designed, both bivalent for BACE1, but (i) one bivalent for hTfR (a chain with the anti-BACE1 VH sequence followed by SEQ ID NO:407 from N- to C-terminus, a chain with the anti-BACE1 VL sequence followed by SEQ ID NO:410 from N- to C-terminus, and a chain with sequence SEQ ID NO:411) and (ii) the other monovalent for hTfR (a chain with the anti-BACE1 VH sequence followed by SEQ ID NO:408 from N- to C-terminus, a chain with the anti-BACE1 VH sequence followed by SEQ ID NO:409 from N- to C-terminus, a chain with the anti-BACE1 VL sequence followed by SEQ ID NO:410 from N- to C-terminus, and a chain with sequence SEQ ID NO:411) (**FIG. 7**). Mutations in the anti-BACE1 Fab (CH1: L128R, K147R; CL: Q124E, V133Q T178E) and the anti-TfR Fab (CH1: L145Q, K147E, S181E; CL: T129R,

T178R, T180Q) were used to promote proper light-chain pairing. Mutations in the Fc region were used to reduce effector function (L234A, L235A, P329G) and to promote hetero-Fc formation for the monovalent anti-TfR bispecific (knob-in-hole). Antibodies were transiently expressed in CHO and purified by standard affinity chromatography (MabSelect SuRe, elution in phosphate pH 2.8), followed by size exclusion chromatography (Superdex 200). To evaluate *in vivo* PKPD behavior of these antibodies, hTfR KI mice were administered a 50 mg/kg molar IgG equivalent IV dose of either control hIgG, anti-BACE1, anti-BACE1/ANTIBODY X bivalent, or anti-BACE1/ANTIBODY X monovalent (by tail-vein injection, four animals per group). After either 1, 3, or 7 days, animals were anesthetized with ketamine/xylazine IP, blood samples were collected by cardiac puncture, vasculature was perfused with ice-cold PBS/heparin, and brains were harvested, as described above. Frozen brain hemispheres were pulverized and separately analyzed for total hIgG and amyloid beta 1-40 (Ab40) by an MSD immunoassay (V-PLEX A β Peptide Panel 1 4G8 Kit).

[00254] The two anti-BACE1/TfR bispecifics cleared from plasma much faster than the anti-BACE1 or control hIgG, and the plasma concentrations of the bispecific that is monovalent for TfR were lower than for the bispecific that is bivalent for TfR (FIG. 8A). However, the brain levels of the bispecific antibodies were higher than the monospecific or control antibodies, with the monovalent TfR binder detected at higher levels than the bivalent TfR binder (FIG. 8B). Both bispecifics had sustained brain exposure over control out to seven days. Ab40 levels in brain lysate were substantially reduced (~50% as expected for BACE1 inhibition) by both the anti-BACE1/TfR bispecific antibodies, and this pharmacodynamic effect was also sustained out to seven days post IV administration (FIG. 8C).

EXAMPLE 8: ANTIBODY X OLIGONUCLEOTIDE

[00255] To demonstrate that ANTIBODY X can enhance the CNS activity of oligonucleotides from IV administration, a bivalent form of ANTIBODY X (comprising SEQ ID NO:401 and SEQ ID NO:412) and a monovalent form of ANTIBODY X (comprising SEQ ID NO:401, SEQ ID NO:413, and SEQ ID NO:414), as well as a control hIgG, with a cysteine modification for conjugation (S442C) were generated. A 3–10–3 cEt gapmer antisense oligonucleotide (ASO) targeting the metastasis associated lung adenocarcinoma transcript 1

(Malat-1) with potency of ED50 = 0.3 $\mu\text{mol/kg/week}$ for reducing Malat-1 mRNA in mouse liver following three weekly doses was synthesized (WuXi) and functionalized for conjugation by reaction with maleimide-PEG₂-succinimidyl ester (SM(PEG)₂, Thermo 22103). Antibodies were reduced (1mM TCEP, 60-90 minutes room temperature), loaded on a Mabselect SuRe column, reoxidized (2mM DHAA, 2 hours room temperature), washed, eluted (25mM NaPi pH 2.8, 0.1M NaCl) and neutralized to pH 5.0, then reacted at ~1:1 molar equivalents with maleimide-ASO. Conjugates with 1:1 antibody(Ab)/ASO ratio were isolated by anion exchange chromatography (TMAE column, elution with 10mM NaPi pH7.0, 0.6M NaCl), confirmed by SDS-PAGE and UV spectrophotometry. Final pools were dialyzed into PBS and protein concentrations were determined by the BCA assay (Thermo A53226).

[00256] To confirm brain biodistribution properties of the Ab:ASO conjugates, hTfR KI mice were dosed at 20 mg/kg IgG:0.75 mg/kg ASO molar equivalent, by tail vein injection of either free ASO, control hIgG-ASO, bivalent ANTIBODY X-ASO, or monovalent ANTIBODY X-ASO. After 24 hours, mice were sacrificed with cardiac perfusion and brain and serum samples were collected (as described above). Total antibody concentrations in serum and brain lysate were quantified by hIgG MSD immunoassay. To quantify free ASO concentrations in brain, brain homogenates were extracted by hybridization using magnetic streptavidin beads coated with biotinylated DNA probe complementary to the Malat1 ASO. The ASO in tissue homogenates were captured on the beads, washed, and then released to a new plate using heat denaturation. The extracted ASO was quantified by LC-MS/MS using a C18 reverse-phase column and multiple reaction monitoring.

[00257] In mice dosed with either bivalent or monovalent ANTIBODY X-ASO conjugates, the antibody was detected in serum at ~4x lower levels than in mice dosed with control hIgG-ASO conjugates (**FIG. 9A**). However, in brain lysate, the bivalent ANTIBODY X was ~5x higher and the monovalent ANTIBODY X was ~9x higher than control hIgG (**FIG. 9B**). Similarly, free ASO in brain lysate was detected for animals dosed with either ANTIBODY X-ASO, at higher levels for the monovalent than the bivalent antibody, but was below the limit of quantitation in brains of animals dosed with ASO alone or control hIgG-ASO (**FIG. 9C**).

[00258] To elicit a pharmacodynamic response in knockdown of Malat1 mRNA, a multi-dose study was conducted, dosing conjugates at 40 mg/kg IgG:1.5 mg/kg ASO molar equivalents

four times weekly IV in hTfR KI mice. To prevent an anti-drug antibody response in the mice, anti-mouse CD4 GK1.5 (BioXcell BP003-1) was administered at 20 mg/kg IP four days before the first and third doses of the test articles. Mice were sacrificed three days after the last dose, harvesting brain hemispheres, spinal cord, tibialis anterior, and liver, flash frozen and stored for analysis. A brain hemisphere from each animal was analyzed for free ASO accumulation by LC-MS/MS, as described above. The tissues were homogenized in TRIzol reagent (Qiagen). Total RNA was then isolated using PureLink RNA Mini Kit (Invitrogen) combined with in-column DNA digestion with DNase I (Qiagen). Reverse transcription was performed using SuperScript VILO Master Mix (Invitrogen) to generate cDNA. The QX200 AutoDG Droplet Digital PCR System (Bio-Rad) was used to detect and quantify transcripts of *Malat1* and house-keeping gene according to the manufacturer's instructions. The QX200 Automated Droplet Generator (Bio-Rad) provided microdroplet generation, and PCR was performed on the T100 Thermal Cycler (Bio-Rad). Plates were read on the QX200 Droplet Reader (Bio-Rad) and analyzed using QuantaSoft Analysis Software (Bio-Rad) to quantify copies of transcripts per well. The expression level of *Malat1* was normalized to that of *Actb*, and this was further normalized to the level in vehicle treated animals.

[00259] Similar to the results from the single-dose study, ANTIBODY X delivered ASO to the brain at much higher levels than from dosing ASO alone or control hIgG-ASO conjugate (**FIG. 9D**). ASO accumulated to ~9nM in brain lysate from administration of the bivalent ANTIBODY X-ASO conjugate and to ~18nM from the monovalent ANTIBODY X-ASO conjugate. Correspondingly, while no reduction in *Malat1* mRNA in brain was observed from dosing ASO alone or control hIgG-ASO conjugate, relative to DPBS vehicle dosed mice, the bivalent and monovalent ANTIBODY X-ASO conjugates lowered *Malat1* mRNA by ~40% and ~50%, respectively (**FIG. 9E**). A similar trend was observed in spinal cord, where *Malat1* knockdown was ~50% and ~60% from administering the bivalent and monovalent ANTIBODY X-ASO conjugates, respectively. *Malat1* knockdown was also observed in peripheral tissues, such as muscle (tibialis anterior) where dosing ASO alone had limited effect, and in liver where dosing ASO alone resulted in greater knockdown than Ab-ASO conjugates. ANTIBODY X is effective at delivering conjugated ASO to the CNS from peripheral administration, at levels sufficient to induce target mRNA knockdown, while also delivering to other therapeutically relevant tissues without increasing delivery to liver (where toxicity may arise).

[00260] To assess whether ANTIBODY X could also deliver effective concentrations of short-interfering ribonucleic acid (siRNA) to the CNS from IV administration, the monovalent form of ANTIBODY X (comprising SEQ ID NO:401, SEQ ID NO:413, and SEQ ID NO:414), as well as a control hIgG, with a cysteine modification for conjugation (S442C) were conjugated to an siRNA targeting hypoxanthine-guanine phosphoribosyltransferase (HPRT). This siRNA has a mixed phosphorothioate-phosphate backbone, mixed 2'-O-methyl and 2'-fluoro modifications, (CH₂)₆NH₂ on the sense strand 3' end, and vinylphosphonate on the antisense 5' end. The sense strand was reacted with *N*-Succinimidyl 3-Maleimidopropionate hetero-bifunctional crosslinker (BMPS, TCI S0427), annealed with the antisense strand, and conjugated to control hIgG (S442C) or the monovalent ANTIBODY X (S442C) as described above. Antibody-siRNA 1:1 conjugates were isolated by anion exchange chromatography.

[00261] Cellular activity of antibody-siRNA conjugates was tested in HEK293T cells, for HPRT mRNA knock-down, and in hTfR-MDCKII transwells for transcytosis. Conjugates or siRNA alone at various concentrations (up to 6000 nM siRNA) were incubated with HEK293T (5000 cells/well) for 72h in DMEM + 10% FBS, then washed in cold PBS, lysed and analyzed for HPRT (Hs01003270_g1, Invitrogen) normalized by ACTB (4333762T, Invitrogen) mRNA by qPCR (TaqMan 4399002, Invitrogen), relative to untreated cells or cells treated with siRNA and transfection reagent (Lipofectamine RNAiMax, Invitrogen). Unconjugated siRNA treatment resulted in knock-down of HPRT mRNA, but with low potency (IC₅₀ ~ 1300 nM; **FIG. 16A**). Whereas conjugation to control hIgG did not affect siRNA activity in this cellular assay (IC₅₀ ~ 1200 nM), conjugation to monovalent ANTIBODY X increased potency of HPRT knock-down in HEK293T over 40-fold (IC₅₀ ~ 30 nM). This demonstrates that targeting human transferrin receptor with monovalent ANTIBODY X is sufficient to enhance functional delivery of siRNA into cells in vitro.

[00262] To evaluate the impact of siRNA conjugation on the transferrin receptor mediated transcellular trafficking of ANTIBODY X, antibody-siRNA conjugates were compared to unconjugated antibody in hTfR-cell transcytosis. Madin-Darby canine kidney II cells (MDCK II, ECACC 00062107) were stably transduced lentiviral particles encoding the human TfR1 gene and expression of human TfR1 was validated using anti-TfR antibodies in flow cytometry. MDCKII/hTfR cells were plated onto Corning 0.4 micron pore transwell inserts with 25,000 cells per insert in complete medium, cultured for five days, and treated with antibodies or

antibody-siRNA conjugates at 100 nM in the top well. After two days of treatment, samples were collected from top and bottom chambers and analyzed for concentration of the test article using commonly available anti-human IgG reagents in a mesoscale discovery assay system. Very little control hIgG accumulated in the transwell bottom chamber (<2 nM), but monovalent ANTIBODY X was detected at ~100x higher concentrations, suggesting active TfR1-mediated cellular transport occurred (**FIG. 16B**). siRNA conjugated monovalent ANTIBODY X also showed active transport, but at roughly half the level of the unconjugated monovalent ANTIBODY X. This indicates that siRNA conjugation has a modest effect in limiting cellular transport of ANTIBODY X.

- 10 **[00263]** The pharmacodynamic activity of the monovalent ANTIBODY X-siRNA conjugate in knocking down HPRT mRNA in the CNS following IV administration was tested in hTfR1 KI mice. Four weekly doses of siRNA or antibody-siRNA conjugates, at 4 mg/kg siRNA or 40 mg/kg hIgG molar equivalents were IV administered, with anti-CD4 20 mg/kg IP prior to the first and third doses to suppress anti-drug antibodies. Three days after the last dose, mice
- 15 were sacrificed, brains were harvested, total RNA isolated, and HPRT mRNA levels relative to ACTB mRNA were measured by droplet digital PCR (ddPCR). Neither siRNA alone nor control IgG-siRNA conjugate treatments resulted in a significant change in HPRT mRNA level in brain. However, monovalent ANTIBODY X-siRNA treatment results in a 60% knockdown of HPRT mRNA (**FIG. 16C**). This suggests that ANTIBODY X can transport oligonucleotides,
- 20 including ASO and siRNA, into the CNS from systemic administration, at sufficient concentrations and to the right compartment within brain cells to induce significant target knock-down.

EXAMPLE 9: ANTIBODY X AFFINITY MODULATION

- 25 **[00264]** Affinity variants of ANTIBODY X were designed. The structural analysis of the ANTIBODY X Fab/TfR1/Tf ternary complex identified specific residues at the ANTIBODY X paratope that could be modified to optimize its affinity for TfR1 (**FIG. 10**). For the reduced affinity designs, mutation sites were selected that trim back or remove favorable contacts with TfR1 by substituting with smaller side chains on the ANTIBODY X paratope. These point
- 30 mutations included S40A, S60A, Y67S, Y110S, and R111S of heavy chain (according to AHo

numbering of SEQ ID NO:100), and Y40N, Y57S, and N69S from the light chain (according to AHo numbering of SEQ ID NO:200). These mutations eliminate H-bonds and vander waal interactions between ANTIBODY X and Tfr1 observed in the cryoEM structure. Another approach was used in designing mutations that increase bulk in the side chains of ANTIBODY X at antigen-binding interface to decrease overall complementarity with Tfr1. These mutations consisted of HC: A112Y (according to AHo numbering of SEQ ID NO:100), and LC: S39N (according to AHo numbering of SEQ ID NO:200). The above strategies utilized amino acid frequencies from all antibody V regions in the NCBI Protein database to ensure that the point mutations were not unusual.

10 **[00265]** Single point mutants of ANTIBODY X were cloned as bivalent aglycosylated hIgG1, expressed in CHO, and purified by standard methods as described above. Bivalent affinity for cell surface human and cynomolgus monkey Tfr1 (at 4 °C) was assessed by flow cytometry (**FIG. 11**). While some mutations had little effect on binding (e.g., VH-S40A, VH-A112Y) relative to ANTIBODY X, a wide range of apparent affinities was observed for the set of ANTIBODY X variants (EC_{50} ~0.5 to >300 nM; **Table 10**). To quantify the impact of the select ANTIBODY X mutations on monovalent affinity, the ANTIBODY X variants were digested into Fab fragments by first incubating antibodies at 5 mg/ml for 4 hours at 37 °C with papain (Roche 108014; 10ug per mg of antibody) in buffer containing 20 mM cysteine, quenching with E-64 protease inhibitor, then removing the Fc fragments and undigested antibody by passing digests over MabSelect SuRe resin, and buffer exchanging into PBS. Monovalent affinity for human and cynomolgus monkey Tfr ECD was determined by surface plasmon resonance, capturing the histidine tagged Tfr1 ECD proteins at ~50RU on an anti-His capture chip in a Biacore 8K+ (Cytiva), and injecting Fab fragments at 7.8 – 8000 nM (4-fold dilution series), analyzing the binding response using a 1:1 kinetics model (Biacore Insight Evaluation Software, Cytiva) (**FIG. 12**). A wide range of monovalent affinities (equilibrium dissociation constants, K_D) and corresponding dissociation rate constants (k_d) were observed among the ANTIBODY X mutants (**Table 10**), from the VL-A112Y variant with a slightly stronger affinity (K_D = 26nM) than parent ANTIBODY X (K_D = 40nM), to the VH-R101S mutant with K_D ~ 5600 nM and the VH-Y110S mutant where binding was observed but with affinity too weak to quantify. The affinity variants generally maintained the good human-cyno affinity match (<3-

fold) of parent ANTIBODY X, although the VH-S60A mutation seemed to improve this species cross-reactivity even further.

Table 10.

Antibody / Variant	Bivalent mAb Tfr-CHO binding		Fab affinity and kinetics Surface Plasmon Resonance						Bivalent mAb Transcytosis	Bivalent mAb Tfr degradation
	hu Tfr EC ₅₀ (nM)	cy Tfr EC ₅₀ (nM)	hu Tfr k _a (/M.s)	hu Tfr k _d (/s)	hu Tfr K _D (nM)	cy Tfr k _a (/M.s)	cy Tfr k _d (/s)	cy Tfr K _D (nM)	hu Tfr MDCKII fold / control	Caco2 Tfr1 % control
ANTIBODY X	0.5	1.1	5.2 x10 ⁵	2.1 x10 ⁻²	40	3.7 x10 ⁵	3.7 x10 ⁻²	99	2.9	31
VH-S40A	0.8	1.7	4.9 x10 ⁵	1.6 x10 ⁻²	33	3.3 x10 ⁵	3.1 x10 ⁻²	93	4.3	29
VH-A112Y	1.0	1.6	2.5 x10 ⁵	6.4 x10 ⁻³	26	1.4 x10 ⁵	8.0 x10 ⁻³	59	n.d.	25
VL-N69S	1.4	3.5	4.6 x10 ⁵	3.4 x10 ⁻²	73	3.4 x10 ⁵	6.7 x10 ⁻²	200	5.9	31
VH-S60A	1.7	1.1	5.2 x10 ⁵	3.4 x10 ⁻²	64	3.6 x10 ⁵	2.8 x10 ⁻²	78	6.3	31
VL-S39N	6.0	23	3.4 x10 ⁵	7.9 x10 ⁻²	230	2.5 x10 ⁵	1.5 x10 ⁻¹	610	7.8	43
VL-Y57S	13	67	1.7 x10 ⁵	1.3 x10 ⁻¹	790	1.3 x10 ⁵	2.5 x10 ⁻¹	1900	6.5	61
VH-Y67S	16	61	1.8 x10 ⁵	2.4 x10 ⁻¹	1400	1.9 x10 ⁵	5.6 x10 ⁻¹	3000	4.4	59
VL-Y40N	~50	~300	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.7	64
VH-R111S	~100	~300	1.2 x10 ⁵	6.8 x10 ⁻¹	5600	--	--	>8000	1.6	88
VH-Y110S	>1000	>1000	--	--	>8000	--	--	>8000	1.3	96

5

[00266] An additional single point mutant of ANTIBODY X (VL-S83A) was generated and tested for binding to human and cyno Tfr1, as described above. The affinity and corresponding binding kinetics (association rate constant (k_a) and dissociation rate constant (k_d)) observed with VL-S83A Fab were K_D = 52nM, k_a = 6.6 x10⁵ /M /s, and k_d = 3.5 x10⁻² /s for human Tfr1, and K_D = 122 nM, k_a = 6.0 x10⁵ /M /s, and k_d = 7.2 x10⁻² /s for cynomolgus Tfr1.

10

[00267] To assess the impact of affinity modulation of ANTIBODY X on cellular antibody transport, antibodies were tested in a Madin-Darby canine kidney II (MDCK II) cell model of human TfR1 mediated transcytosis. MDCK II cells (ECACC 00062107) were stably transduced with VSV-G pseudotyped lentiviral particles encoding a human TfR1 gene under a human EF1a promoter with an IRES-Puromycin resistance cassette. Cells were selected for puromycin resistance to produce a MDCKII/hTfR cell line. Expression of human TfR1 was validated using anti-TfR antibodies in flow cytometry.

[00268] MDCKII/hTfR cells were plated onto Corning 0.4 micron pore transwell inserts with 25,000 cells per insert in complete medium, 0.25ml in the insert and 1 ml in the bottom chamber, and kept in an incubator at 37 °C and 5% CO₂. On day 4 the bottom media was fully replaced and half of the insert media was replaced with fresh media. On day 5, test article was added to the top well at 100 nM. On day 7, samples were collected from top and bottom chambers and analyzed for concentration of the test article. Quantitation of test article was performed using commonly available anti-human IgG reagents in a mesoscale discovery assay system, with results extrapolated from a titrated standard curve of the same test article.

[00269] ANTIBODY X weakened affinity variants generally showed improved cellular transport relative to parent ANTIBODY X (Table 10; FIGS. 13A-13B), up to ~8x control hIgG, but with a bell-shaped dependence on affinity. The optimal range of affinities for maximal cellular transcytosis, in this assay, was EC₅₀ ~ 1-10 nM for bivalent binding of cell surface TfR1 (at 4 °C; FIG. 13A) or a monovalent affinity of K_D ~ 100-1000 nM for recombinant hTfR1 ECD (at 25 °C; FIG. 13B). The weaker affinity variants (e.g., VH-Y67S and VH-R111S) showed reduced transcytosis, likely due to incomplete engagement of hTfR1 at the concentration tested in this assay (100nM) but may still transport at the BBB *in vivo* when antibody concentrations in blood are higher.

[00270] To investigate the impact of affinity dematuration on *in vivo* biodistribution, huTfR1 KI mice were administered a 20 mg/kg (or molar IgG equivalent) IV dose of ANTIBODY X variants or control hIgG. Whole blood, serum, and perfused brain hemispheres were harvested either 1 or 7 days later, as described above. Total hIgG in serum and brain lysate were quantified by MSD immunoassay and a complete blood cell count was recorded for each mouse.

[00271] At one day post IV administration, the biodistribution of bivalent and monovalent forms of ANTIBODY X ($K_D = 40$ nM) were compared to bivalent ANTIBODY X affinity variants VL-S39N ($K_D = 230$ nM), VL-Y57S ($K_D = 790$ nM), and VH-Y67S ($K_D = 1400$ nM). The monovalent ANTIBODY X had the lowest levels in serum but the highest levels in brain, and minimal reticulocyte loss (**FIG. 14**). The bivalent ANTIBODY X had higher serum levels than monovalent ANTIBODY X and serum levels were further improved for variants with weaker affinity to hTfR1. However, the brain levels of all bivalent antibodies was roughly half that of the monovalent ANTIBODY X, independent of affinity. There was a trend toward less severe reticulocyte depletion with weaker affinity for hTfR1.

[00272] At seven days post IV administration, there was a clear effect of weakened affinity for TfR on the persistence of antibody in serum and brain, while also minimizing impact on reticulocytes (**FIG. 14**). The bivalent ANTIBODY X was at ~1000x lower levels in serum than control hIgG, but the VL-S39N, VL-Y57S, VH-Y67S, and VH-R111S were all at much higher serum levels, in rank-order with affinity. The bivalent VH-R111S variant, with monovalent $K_D \sim 5600$ nM for TfR1, was only ~2x lower than the control hIgG in serum. The three weakest affinity variants were also at elevated levels in brain lysate, 4-6x higher than control hIgG, 7 days after IV administration, demonstrating the value of weakened affinity for TfR in sustaining brain exposure. Furthermore, the weakest affinity variant, VH-R111S also had no significant effect on reticulocyte counts at this 7 day time point, despite still being present in serum and being functional in TfR1-mediated brain uptake.

[00273] Understanding the effect of anti-TfR antibodies on cellular trafficking and stability of expression of TfR1 is important to gauge the safety and durability of TfR1-mediated brain delivery of therapeutics. To quantify these effects in an *in vitro* cellular system, Caco2 cells (ECACC 86010202) were seeded at 30,000 cells/well in a 12-well plate, cultured for 4 days, then incubated with ANTIBODY X or control hIgG at 1000nM for 24 hours. Following antibody treatment, cells were lysed in RIPA buffer (Cell Signaling Technology 9806S) with phosphatase and protease inhibitors (PhosSTOP and cOmplete; Sigma) on ice for 30 minutes, centrifuged at 13,000rpm for 15 minutes, and supernatant was collected and analyzed for total protein by BCA (Pierce 23227). Lysates were then normalized to 100 ug/mL protein and analyzed by capillary Western Blot (Jess, ProteinSimple), probing for TfR1 (H68.4; Thermo 13-6800) and beta-actin (MAB8929; R&D Systems) with chemiluminescent secondary antibodies.

[00274] ANTIBODY X (Fab-Fc bivalent) treatment resulted in substantially less TfR1 in cell lysate than treatment with control hIgG (**FIG. 15A**). This loss of TfR1 was completely reversed by inclusion of the ATPase inhibitor, bafilomycin (100nM), during treatment, suggesting that ANTIBODY X leads to lysosomal trafficking and degradation of TfR1. To understand how antibody format affects TfR1 degradation, cell treatment with monovalent Fab-Fc, bivalent Fc-Fab, and monovalent Fc-Fab ANTIBODY X formats were also tested for TfR1 levels and compared to the effect of the bivalent Fab-Fc formatted ANTIBODY X. The monovalent antibody treatments resulted in much higher levels of TfR1 retained in cell lysate as compared to the bivalent antibody formats. The increased lysosomal trafficking and loss of TfR1 by bivalent antibodies may be due to either receptor cross-linking or increased avidity due to avidity. To examine whether reducing affinity through mutagenesis can minimize antibody-induced TfR1 loss, the ANTIBODY X affinity variants (in bivalent format) were tested for cellular TfR1 degradation. Indeed, the extent of TfR1 degradation by ANTIBODY X affinity variants correlated well with the EC₅₀ of cell surface TfR1 binding (**Table 10; FIG. 15B**). Moreover, the format variants and affinity variants of ANTIBODY X all seemed to follow the same correlation of TfR1 degradation with cellular TfR1 binding affinity ($R^2 = 0.89$), suggesting that cell surface TfR binding is a sufficient predictor of lysosomal trafficking, independent of valency.

[00275] *In vivo*, inducing TfR1 degradation could have consequences for sustained transport of antibodies across the BBB, but also on cellular function and survival. Reticulocyte depletion is a known consequence of systemic administration of anti-TfR1 antibodies, seems to happen even with minimal Fc effector function, but is dependent on TfR1 affinity (**FIG. 14**). Comparing the reticulocyte loss in vivo to the TfR1 degradation induced in vitro for select ANTIBODY X variants (**FIG. 15C**), suggests that there may be correlation supporting TfR1 degradation as a mechanism for reticulocyte depletion and that either monovalent or reduced-affinity variants can be used without significant deleterious effects.

EXAMPLE 10: ANTIBODY X AFFINITY REFINEMENT BY COMBINATORIAL MUTAGENESIS

[00276] Given the impact of affinity modulation on the functional properties of anti-TfR ANTIBODY X, a second set of variants was designed for refined binding properties. Guided by
5 the first round of ANTIBODY X mutagenesis, the second set incorporated combination of the VH-S60A mutation that resulted in closely matched affinities for human and cynomolgus monkey TfR1 ($K_D = 64$ and 78 nM, respectively), with the VL-S39N, VL-Y57S, VH-Y67S, and VH-R111S mutations that weakened affinity for human TfR1 across the range $K_D \sim 200$ - 6000 nM. These double mutants were generated as bivalent and monovalent (Fab fragment)
10 antibodies. Bivalent antibodies were tested for binding CHO cells expressing human or cynomolgus monkey TfR1 by flow cytometry, whereas affinity and kinetics of Fab fragments binding human or cynomolgus monkey TfR1 ECD were tested by surface plasmon resonance, as described in earlier examples. The results are shown in **Table 11**.

[00277] All four double mutant bivalent antibodies have cell surface EC_{50} of binding that
15 is less than 2-fold different between human and cynomolgus monkey TfR1, ranging from ~ 50 nM to ~ 250 nM (at 4°C). For the VH-S60A / VL-S39N and the VH-S60A / VL-Y57S double mutants, the Fab affinities (K_D by SPR at 25°C) are also very closely matched for human and cynomolgus TfR1 ectodomain. However, the affinities of the VH-S60A/Y67S and VH-S60A/R111S double mutant Fabs could not be measured accurately to both species of TfR1
20 ECD, as binding appeared to be well below saturation at the top concentration tested in the assay (6000 nM, due to the limitations of non-specific binding). An additional double mutant bivalent antibody (VH-S60A/S61A) was assessed as described in this example. This double mutant resulted in weaker human affinity than cyno affinity: $K_D = 235$ nM (hu) and 127 nM (cy) for VH-S60A/S61A Fab versus $K_D = 64$ nM (hu) and 78 nM (cy) for VH-S60A Fab.

25 [00278] Overall, the combination of the VH-S60A mutation for matching affinity between species and additional mutations that vary affinity without modulating species cross-reactivity were applied to generate this refined set of weakened affinity antibodies with improved properties relative to the single point mutant ANTIBODY X variants.

Table 11. Binding of ANTIBODY X double point mutation variants to human and cynomolgus monkey TfR1.

Antibody Variant	Bivalent mAb TfR-CHO binding		Monovalent Fab TfR1 ECD affinity	
	hu TfR EC ₅₀ (nM)	cy TfR EC ₅₀ (nM)	hu TfR K _D (nM)	cy TfR K _D (nM)
VH-S60A (SEQ ID NO: 102) / VL-S39N (SEQ ID NO: 201)	48	82	550	730
VH-S60A (SEQ ID NO: 102) / VL-Y57S (SEQ ID NO: 203)	86	130	~1300	~1500
VH-S60A/Y67S (SEQ ID NO: 107)	120	150	~3000	n.d.
VH-S60A/R111S (SEQ ID NO: 108)	170	~250	n.d.	n.d.

EXAMPLE 11: AFFINITY VARIANTS OF ANTIBODY X HAVE IMPROVED PHARMACOKINETICS AND RETICULOCYTE DEPLETION EFFECTS IN BISPECIFIC FORMAT

[00279] In short-term biodistribution studies, ANTIBODY X shows superior brain exposure when in monovalent format, either as a monospecific antibody (FIG. 14), incorporated into a bispecific antibody (FIG. 8), or as an oligonucleotide conjugate (FIG. 9), compared to corresponding applications with bivalent ANTIBODY X (even across a broad range of bivalent affinities). To examine whether anti-TfR antibody mediated delivery to the CNS, and resulting brain exposure, can be further optimized with monovalent TfR1 targeting, affinity variants of ANTIBODY X in monovalent format were incorporated into a bispecific antibody. A therapeutic ANTIBODY Z was combined with a Fab fragment of an ANTIBODY X variant as a 2:1 bispecific antibody (bivalent ANTIBODY Z: monovalent ANTIBODY X; see FIG. 18A). Four versions of this bispecific antibody were designed (all incorporating bivalent ANTIBODY Z), where Fab fragments of each ANTIBODY X variant were genetically fused (via a 2xG4S polypeptide chain) to the C-terminus of one heavy chain of the non-targeting bivalent ANTIBODY Z (using additional constant domain mutations to ensure proper light chain pairing and Fc heterodimerization). These antibodies were administered intravenously in hTfR KI mice at 3 mg/kg molar IgG equivalent to investigate the relationship between affinity and biodistribution when targeting hTfR1 monovalently at a sub-saturating dose. In addition, since these bispecifics were designed with full effector function (glycosylated hIgG1 Fc), the impact on reticulocyte dynamics was closely monitored. Two terminal cohorts of mice were analyzed

for each antibody, one sacrificed one day and the other sacrificed seven days after IV administration, and following cardiac perfusion, brain samples were collected, lysed and analyzed for hIgG uptake.

[00280] Serum pharmacokinetics for bispecific antibodies incorporating ANTIBODY X variant Fab fragments were highly dependent on the affinity for hTfR1. Clearance rate increased in proportion to TfR1 affinity and was nearly equivalent to that of the mono-specific hIgG1 control for the weakest bispecific variant (VL-Y57S) (**FIG. 18B; Table 12**). Brain exposure for these bispecific antibodies followed a more complex dependence on TfR1 affinity. Short-term brain exposure, assessed at one day post IV administration, decreased with decreasing affinity to TfR1, whereas long-term brain exposure assessed at one week post IV administration was highest for the VH-S60A/VL-S39N variant with intermediate affinity to TfR1 (**FIG. 18C**). To estimate overall brain exposure, the area under the curve (AUC) for serum exposure was calculated and multiplied by the average brain:serum ratio at day 1 and day 7, resulting in the approximate brain AUC values for each bispecific antibody variant (**Table 12**). Finally, the different bispecific antibody variants had differential impact on circulating reticulocytes, with the higher affinity variants showing more depletion of reticulocytes at day 1 and higher rebound (elevated reticulocytes) at day 7, relative to weaker affinity variants or the hIgG control antibody that does not bind TfR1 (**FIG. 18D**). This analysis supports the idea that ANTIBODY X variants have different brain exposure profiles that can be utilized depending on the pharmacological application, and that the choice of variant could also help mitigate the impact of these antibodies on hematology.

Table 12. Biodistribution and reticulocyte depletion properties of 2:1 bispecific antibodies incorporating monovalent ANTIBODY X variants.

2:1 bsAb variant	K _D (nM) huTfR	K _D (nM) cyTfR	Serum CL (mL/day/kg)	Brain AUC (nM*day)	Reticulocyte Depletion (24h)
hIgG control	-	-	15.1	9	0
VH-S60A	69	80	70.5	32	-60%
VL-S39N	232	611	46.5	30	-43%
VH-S60A/VL-S39N	571	592	21.4	46	-20%
VL-Y57S	787	1910	16.2	29	-3%

OTHER EMBODIMENTS

[00281] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

5

WHAT IS CLAIMED IS:

1. An antibody that binds to human transferrin receptor, comprising a heavy chain variable region (VH) comprising VH complementarity determining region (CDR)1, VH CDR2, and VH CDR3, and a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3, wherein:
- 5 the VH CDR1 comprises the amino acid sequence GFTFSSYX₁MN (SEQ ID NO:18) or the amino acid sequence SYX₁MN (SEQ ID NO:26), wherein X₁ is S or A;
- the VH CDR2 comprises the amino acid sequence SISX₂SSSX₃IYYADSVKG (SEQ ID NO:19), wherein X₂ is S or A, and wherein X₃ is Y or S; and
- 10 the VH CDR3 comprises the amino acid sequence KX₄X₅X₆GDFDY (SEQ ID NO:20), wherein X₄ is Y or S, wherein X₅ is R or S, and wherein X₆ is A or Y;
- the VL CDR1 comprises the amino acid sequence RASQSVSSX₇X₈LA (SEQ ID NO:21), wherein X₇ is S or N, and wherein X₈ is Y or N;
- the VL CDR2 comprises the amino acid sequence GASX₉RAT (SEQ ID NO:22),
- 15 wherein X₉ is N or S; and
- the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8).
2. The antibody of claim 1, wherein at least one of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1,
- 20 and any of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3 that are not selected from the mutant CDRs depicted in Table 1 are selected from the parental CDRs depicted in Table 1.
3. The antibody of claim 1, wherein one of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3 is selected from the mutant CDRs depicted in Table 1 and five
- 25 of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1.
4. The antibody of claim 1, wherein two of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and
- 30

four of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1.

5 5. The antibody of claim 1, wherein three of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and three of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1.

10 6. The antibody of claim 1, wherein four of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and two of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the parental CDRs depicted in Table 1.

15 7. The antibody of claim 1, wherein five of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 are selected from the mutant CDRs depicted in Table 1 and one of VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3 is selected from the parental CDRs depicted in Table 1.

20 8. The antibody of claim 1, wherein:
the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8); or

25 the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8).

30

9. The antibody of claim 1, wherein:

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
5 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence GFTFSSYAMN (SEQ ID NO:9); the
10 VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

15 the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
20 the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
25 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSYIYYADSVKG (SEQ ID NO:10); the
30 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SSSSSSIYYADSVKG (SEQ ID NO:11); the
 5 VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
 10 VH CDR2 comprises the amino acid sequence SSSSSSIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KSRAGDFDY (SEQ ID NO:12); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
 15 VH CDR2 comprises the amino acid sequence SSSSSSIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
 20 the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SSSSSSIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRYGDFDY (SEQ ID NO:14); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
 25 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSIYYADSVKG (SEQ ID NO:25); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
 30 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the
 5 VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
 10 VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the
 15 VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNLA (SEQ ID NO:16); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises
 20 the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence GFTFSSYSMN (SEQ ID NO:3); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2
 25 comprises the amino acid sequence GASSRAT (SEQ ID NO:17); and the VL CDR3 comprises the amino acid sequence QQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1
 30 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises

the amino acid sequence QQQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence SYAMN (SEQ ID NO:24); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8), wherein the VL comprises a Y57S conservative substitution;

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:11); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KSRAGDFDY (SEQ ID NO:12); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SSSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRYGDFDY (SEQ ID NO:14); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:25); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISASSSYIYYADSVKG (SEQ ID NO:10); the VH CDR3 comprises the amino acid sequence KYSAGDFDY (SEQ ID NO:13); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2

comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNYLA (SEQ ID NO:15); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8);

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSNLA (SEQ ID NO:16); the VL CDR2 comprises the amino acid sequence GASNRAT (SEQ ID NO:7); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8); or

the VH CDR1 comprises the amino acid sequence SYSMN (SEQ ID NO:23); the VH CDR2 comprises the amino acid sequence SISSSSSYIYYADSVKG (SEQ ID NO:4); the VH CDR3 comprises the amino acid sequence KYRAGDFDY (SEQ ID NO:5); the VL CDR1 comprises the amino acid sequence RASQSVSSSYLA (SEQ ID NO:6); the VL CDR2 comprises the amino acid sequence GASSRAT (SEQ ID NO:17); and the VL CDR3 comprises the amino acid sequence QQQSSSPPT (SEQ ID NO:8).

10. The antibody of any one of the preceding claims, wherein:

(i) the VH is at least 80%, 85%, 90%, 95%, 99%, or 100% identical to any one of SEQ ID NOs:100-108; and
(ii) the VL is at least 80%, 85%, 90%, 95%, 99%, or 100% identical to any one of SEQ ID NOs:200-204.

11. The antibody of claim 1, wherein the VH comprises the amino acid sequence of any one of SEQ ID NOs:100-108 and the VL comprises the amino acid sequence of any one of SEQ ID NOs:200-204.

12. The antibody of any one of the preceding claims, wherein the antibody is (a) monovalent and has a monovalent affinity (K_D) for hTfR1 of > 10 nM or is bivalent and has a monovalent affinity (K_D) for hTfR1 of > 100 nM and/or (b) has an off rate (k_d) of $\geq 0.01/s$.

5 13. The antibody of claim 1, wherein:
the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:200.

10 14. The antibody of claim 1, wherein:
the VH comprises the amino acid sequence of SEQ ID NO:101 and the VL comprises the amino acid sequence of SEQ ID NO:200;
the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO: 200;
the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the
15 amino acid sequence of SEQ ID NO:201;
the VH comprises the amino acid sequence of SEQ ID NO:102 and the VL comprises the amino acid sequence of SEQ ID NO:203;
the VH comprises the amino acid sequence of SEQ ID NO:103 and the VL comprises the amino acid sequence of SEQ ID NO:200;
20 the VH comprises the amino acid sequence of SEQ ID NO:104 and the VL comprises the amino acid sequence of SEQ ID NO:200;
the VH comprises the amino acid sequence of SEQ ID NO:105 and the VL comprises the amino acid sequence of SEQ ID NO:200;
the VH comprises the amino acid sequence of SEQ ID NO:106 and the VL comprises the
25 amino acid sequence of SEQ ID NO:200;
the VH comprises the amino acid sequence of SEQ ID NO:107 and the VL comprises the amino acid sequence of SEQ ID NO:200;
the VH comprises the amino acid sequence of SEQ ID NO:108 and the VL comprises the amino acid sequence of SEQ ID NO:200;
30 the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:201;

the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:202;

the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:203; or

5 the VH comprises the amino acid sequence of SEQ ID NO:100 and the VL comprises the amino acid sequence of SEQ ID NO:204.

15 15. The antibody of any one of claims 1 to 14, which is a multispecific antibody, bispecific antibody, single chain antibody, an Fab fragment, an F(ab')₂ fragment, an Fab' fragment, an Fsc fragment, an Fv fragment, an scFv, an sc(Fv)₂, or a diabody.

16. The antibody of any one of claims 1 to 14, comprising a constant heavy chain (CH) domain and a constant light chain (CL) domain.

15 17. The antibody of claim 1, wherein the antibody comprises:
(a) a heavy chain comprising the amino acid sequence set forth in 400, and a light chain comprising the amino acid sequence set forth in 401;
(b) the amino acid sequences set forth in SEQ ID NOs: 401 and 402;
(c) the amino acid sequences set forth in SEQ ID NOs: 401, 404, and 406;
20 (d) the amino acid sequences set forth in SEQ ID NOs:400 and 401;
(e) the amino acid sequences set forth in SEQ ID NOs: 401, 405, and 406; or
(f) the amino acid sequences set forth in SEQ ID NOs: 401 and 403.

25 18. A nucleic acid or nucleic acids encoding the antibody of any one of claims 1 to 17.

19. An expression vector or expression vectors comprising the nucleic acid or nucleic acids of claim 18 operably linked to a promoter.

30 20. An isolated cell comprising the nucleic acid or nucleic acids of claim 18 or the expression vector or expression vectors of claim 19.

21. An isolated cell comprising a first expression vector comprising a first nucleic acid encoding a first polypeptide comprising the VH of the antibody of any one of claims 1 to 17 operably linked to a promoter, and a second expression vector comprising a second nucleic acid encoding a second polypeptide comprising the VL of the antibody of any one of claims 1 to 17 operably linked to a promoter.

22. A method of making the antibody of any one of claims 1 to 17, comprising culturing the cell of claim 20 or 21 and isolating the antibody.

23. A pharmaceutical composition comprising the antibody of any one of claims 1 to 17 and a pharmaceutically acceptable carrier.

24. A conjugate comprising the antibody of any one of claims 1 to 17 and an agent.

25. The conjugate of claim 24, wherein the agent is an antibody, protein, or peptide.

26. The conjugate of claim 24, wherein the agent is an anti-beta amyloid antibody.

27. The conjugate of claim 26, wherein anti-beta amyloid antibody is aducanumab, bapineuzumab, gantenerumab, solanezumab, donanemab, or lecanemab.

28. The conjugate of claim 24, wherein the agent is an anti-tau antibody, an anti-alpha synuclein antibody, an anti-TDP-43 antibody, an anti-LINGO-1 antibody, an anti-LINGO-2 antibody, an anti-LINGO-3 antibody, an anti-LINGO-4 antibody, an anti-TREM2 antibody, or an anti-C9orf72 dipeptide repeat poly-GA antibody.

29. The conjugate of claim 24, wherein the agent is protein.

30. The conjugate of claim 29, wherein the protein is progranulin.

31. The conjugate of claim 24, wherein the agent is an enzyme.

32. The conjugate of claim 31, wherein the enzyme is glucocerebrosidase.

33. The conjugate of any one of claims 24 to 32, wherein the conjugate is a recombinant
5 fusion protein comprising the antibody and the agent.

34. The conjugate of claim 24, wherein the agent is a nucleic acid.

35. The conjugate of claim 34, wherein the nucleic acid is an mRNA, a siRNA, an
10 antisense oligonucleotide, microRNA (miRNA), guide RNA (gRNA), or a phosphoroamidate
morpholino oligomer (PMO).

36. The conjugate of claim 34 or 35, wherein the nucleic acid is linked to the antibody
via a linker.
15

37. The conjugate of claim 24, wherein the agent is a nanoparticle, liposome, or viral
vector.

38. A method of transporting an agent across the blood brain barrier via transcytosis, the
20 method comprising administering to a human subject the conjugate of any one of claims 24 to
37.

39. A method of delivering an agent *in vivo*, the method comprising administering to a
human subject the conjugate of any one of claims 24 to 37.
25

40. The method of claim 39, wherein the human subject has a neurological disorder and
the method delivers the agent to brain tissue.

41. The method of claim 39, wherein the neurological disorder is Alzheimer's disease,
30 Parkinson's disease, frontotemporal dementia, ALS, Huntington's disease, multiple sclerosis,
spinal muscular atrophy, muscular dystrophy, spinal cord injury, stroke, an ophthalmological

condition, acute or chronic optic neuritis, a psychiatric disorder, Tourette's disease brain injury, a brain tumor, or epilepsy.

42. A method of treating Alzheimer's disease in a human subject in need thereof,
5 comprising administering to the human subject a therapeutically effective amount of the conjugate of claim 26 or 27.