

ANTI-TFR1 ANTIBODIES, PREPARATION METHODS AND USES THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] The present application claims priority to International Application No. PCT/CN2023/143182 filed on December 29, 2023, the contents of which are hereby incorporated by reference in its entirety.

Technical Field

[0002] The present disclosure relates to the field of molecular biology and immunology and particularly to anti-TFR1 antibodies or antigen-binding fragments thereof, multispecific antibodies comprising the same, as well as preparation methods and uses thereof.

Background

[0003] With the improvement of people's living span, the percentage of patients with neurological diseases is increasing. Thus, the development of effective neuropharmaceuticals is critically important and urgent.

[0004] Monoclonal antibodies and other biotherapeutics have huge therapeutic potential for the treatment of central nervous system diseases. Unfortunately, many promising candidates fail to show expected effects because of their inability to cross the blood-brain barrier (BBB), a dynamic interface that separates the central nervous system (CNS) from the circulation system, limiting the ability to treat neurological diseases. Under normal condition of human bodies, several essential nutrients and carrier proteins are thought to cross the BBB via receptors expressed on brain endothelial cells through a process known as receptor-mediated transcytosis. Hence, restoring BBB integrity under pathological conditions to maintain brain homeostasis and opening BBB temporarily to allow for the efficient delivery of drugs to the CNS are potential therapeutic options for patients with these disorders.

[0005] Transferrin receptor 1 (TFR1), also known as cluster of differentiation 71 (CD71), is a type II transmembrane glycoprotein highly expressed on brain endothelial cells that binds transferrin (Tf) and plays a critical role in cellular iron uptake through the interaction with iron bound Tf. It is known that iron dissociates from Tf in acidified endosomes and the Tf-TFR1 complex recycles back to the plasma membrane. In this way, it is involved in the control of cellular iron homeostasis.

[0006] Taking advantage of this mechanism, anti-TFR antibodies have been used as shuttle, to deliver target proteins to cross the BBB without affecting iron homeostasis. Currently available anti-TFR1 antibody-based neuropharmaceuticals include pabinafusp alfa (a fusion protein comprising a humanized anti-human transferrin receptor (TFR) antibody and human iduronate-2-sulfatase) for treating CNS symptoms of MPS-II (Hunter syndrome) and trontinemab (a bispecific antibody targeting amyloid beta (A β) and TFR1) for treating Alzheimer's disease.

[0007] So far, there is a lack of a fully human antibody that does not affect the binding of TFR to TF. Moreover, fully human heavy chain antibodies have a lower molecular weight and cross the blood-brain barrier more easily than conventional antibodies.

Summary

[0008] In one aspect, provided is an anti-transferrin receptor 1 (TFR1) antibody or an antigen-binding fragment thereof, wherein the anti-TFR1 antibody or antigen-binding fragment comprises a heavy chain variable domain, wherein the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3, wherein the VH CDR1 comprises an amino acid sequence differing from SEQ ID NO: 8 by addition, deletion or substitution of no more than 2 amino acids; the VH CDR2 comprises an amino acid sequence differing from SEQ ID NO: 16 by addition, deletion or substitution of no more than 2 amino acids; and/or the VH CDR3 comprises an amino acid sequence differing from SEQ ID NO: 24 by addition, deletion or substitution of no more than 2 amino acids.

[0009] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises an amino acid sequence differing from SEQ ID NO: 24 by amino acid addition, deletion or substitution of no more than 2 amino acids. In some preferable embodiments, the VH CDR3 comprises a non-conservative amino acid substitution at a position corresponding to amino acid 2, 4, 5, or 7 of SEQ ID NO: 24. In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 24, 79, 80, 81 or 82.

[0010] In some embodiments, the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101.

[0011] In some embodiments, the heavy chain variable domain comprises: (A) the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101; or (B) an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101; or (C) an amino acid sequence having amino acid addition, deletion and/or substitution of one or more amino acids in comparison with the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101. In some preferable embodiments, the amino acid addition, deletion and/or substitution do/does not occur in a CDR region.

[0012] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment of the present disclosure, (1) binds to human TFR1 and non-human primate TFR1; and/or (2) does not substantially affect the binding of transferrin to TFR1; and/or (3) binds to the transferrin-TFR1 complex.

[0013] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment of the present disclosure is a single domain antibody or a heavy-chain-only antibody and optionally a fully human antibody.

[0014] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment of the present disclosure further comprises an Fc region, and optionally the Fc region comprises one or more modifications to reduce the effector function and/or enhance the FcRn binding.

[0015] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment of the present disclosure comprises a heavy chain having the amino acid sequence of SEQ ID NO: 59, 107, 108, 109 or 110.

[0016] In another aspect, provided is a transferrin receptor 1 (TFR1)-binding agent comprising the anti-TFR1 antibody or antigen-binding fragment of the present disclosure conjugated to an additional molecule.

[0017] In some embodiments, the additional molecule is a therapeutic agent for treating a disease or disorder selected from: cancer, central nervous system cancer, Alzheimer's disease, Parkinson's disease, Huntington's disease, hunter syndrome, schizophrenia, antidepressant, multiple sclerosis, amyotrophic lateral sclerosis, lysosomal storage disease accompanied by encephalopathy, glycogenosis, muscular dystrophy, cerebral ischemia, a prion disease, traumatic central nervous system disorder, viral and bacterial central nervous system disease.

[0018] In some embodiments, the additional molecule is an imaging agent selected from: a radionuclide, biotin, a fluorescent protein, a fluorophore, horseradish peroxidase and alkaline phosphatase.

[0019] In yet another aspect, provided is a multispecific antibody, comprising a first antigen-binding moiety binding to TFR1, wherein the first antigen-binding moiety comprises the heavy chain variable domain as described herein, and a second antigen-binding moiety binding to a second antigen.

[0020] In a particular aspect, provided is a multispecific antibody binding to TFR1 and BACE1 ("anti-TFR1/BACE1 antibody"). In some embodiments, the anti-TFR1/BACE1 antibody comprises a first antigen-binding moiety binding to TFR1 and a second antigen-binding moiety binding to BACE1, wherein the first antigen-binding moiety comprises the heavy chain variable domain as described herein, the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HCDR1, an HCDR2 and an HCDR3 as comprised in the amino acid sequence of SEQ ID NO: 102, and the light chain variable region comprises an LCDR1, an LCDR2 and an LCDR3 as comprised in the amino acid sequence of SEQ ID NO: 104. In some embodiments, the HCDR1 comprises an amino acid sequence of SEQ ID NO: 67, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 73, and the HCDR3 comprises an amino acid sequence of SEQ ID NO: 83; and the LCDR1 comprises an amino acid sequence of SEQ ID NO: 88, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 91, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 95. In some embodiments, the

heavy chain variable region comprises an amino acid sequence of SEQ ID NO: 102; and/or the light chain variable region comprises an amino acid sequence of SEQ ID NO: 104.

[0021] In another particular aspect, provided is a multispecific antibody binding to TFR1 and amyloid beta (A β) (“anti-TFR1/A β antibody”). In some embodiments, the anti-TFR1/A β antibody comprises a first antigen-binding moiety binding to TFR1 and a second antigen-binding moiety binding to A β , wherein the first antigen-binding moiety comprises the heavy chain variable domain as described herein, the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HCDR1, an HCDR2 and an HCDR3 as comprised in the amino acid sequence of SEQ ID NO: 103, and the light chain variable region comprises an LCDR1, an LCDR2 and an LCDR3 as comprised in the amino acid sequence of SEQ ID NO: 105. In some embodiments, the HCDR1 comprises an amino acid sequence of SEQ ID NO: 68, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 74, and the HCDR3 comprises an amino acid sequence of SEQ ID NO: 84; and the LCDR1 comprises an amino acid sequence of SEQ ID NO: 89, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 92, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 96. In some embodiments, the heavy chain variable region comprises an amino acid sequence of SEQ ID NO: 103; and/or the light chain variable region comprises an amino acid sequence of SEQ ID NO: 105.

[0022] In some embodiments, the second antigen-binding moiety comprises a dsFv, an scFv, an scdsFv, a di-scFv, an Fab, an scFab or an F(ab')₂.

[0023] In some embodiments, the multispecific antibody of the present disclosure comprises only one heavy chain variable domain as described herein.

[0024] In some embodiments, the multispecific antibody of the present disclosure further comprises an Fc region, and optionally the Fc region comprises one or more modifications to reduce the effector function and/or enhance the FcRn binding. In some embodiments, the Fc region is a heterodimeric Fc region comprising a first Fc region subunit and a second Fc region subunit, wherein one of the first Fc region subunit and the second Fc region subunit comprises a knob mutation, and the other comprises a hole mutation. In some embodiments, the knob mutation comprises one or more mutations selected from S354C and T366W; and/or the hole mutation comprises one or more mutations selected from Y349C, T366S, L368A and Y407V. In some preferable embodiments, the knob mutation comprises S354C and T366W, and the hole mutation comprises Y349C, T366S, L368A and Y407V.

[0025] In some embodiments, the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region, and the multispecific antibody comprises:

- (a) (1) a first polypeptide comprising the heavy chain variable region, a CH1 domain and a first Fc region subunit, (2) a second polypeptide comprising the light chain variable region and a light chain constant region, and (3) a third polypeptide comprising the heavy chain variable domain and a second Fc region subunit, or

(b) (1) a first polypeptide comprising the heavy chain variable region, a CH1 domain, a first Fc region subunit, an optional peptide linker and the heavy chain variable domain, (2) a second polypeptide comprising the heavy chain variable region, a CH1 domain and a second Fc region subunit, and (3) a third polypeptide and a fourth polypeptide each comprising the light chain variable region and a light chain constant region, wherein the first Fc region subunit and the second Fc region subunit form the heterodimeric Fc region.

[0026] In some embodiments, the anti-TFR1/BACE1 antibody comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 122, a second polypeptide having the amino acid sequence of SEQ ID NO: 113, and a third polypeptide having the amino acid sequence of SEQ ID NO: 129, 130, 131, 132 or 133.

[0027] In some embodiments, the anti-TFR1/A β antibody comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 119, 125, 126, 127 or 128, a second polypeptide having the amino acid sequence of SEQ ID NO: 117, and a third polypeptide and a fourth polypeptide each having the amino acid sequence of SEQ ID NO: 115.

[0028] Further provided is a pharmaceutical composition or a kit comprising the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent or the multispecific antibody of the present disclosure.

[0029] Further provided are a nucleic acid encoding the anti-TFR1 antibody or antigen-binding fragment, or the multispecific antibody of the present disclosure, a vector comprising the nucleic acid of the present disclosure, and a host cell comprising the nucleic acid or the vector of the present disclosure.

[0030] In a further aspect, provided is use of the anti-TFR1 antibody or antigen-binding fragment of the present disclosure in the manufacture of an agent for transporting a compound across the blood-brain barrier.

[0031] In yet another aspect, provided is use of the anti-TFR1 antibody or antigen-binding fragment of the present disclosure in the manufacture of a medicament for treating cancer.

[0032] Further provided is use of the anti-TFR1 antibody or antigen-binding fragment of the present disclosure in the manufacture of a medicament for treating a neurological disease or disorder, wherein the anti-TFR1 antibody or antigen-binding fragment is conjugated to a therapeutic agent for treating the neurological disease or disorder.

[0033] In another aspect, provided is use of the multispecific antibody of the present disclosure in the manufacture of a medicament for treating a neurological disease or disorder; preferably, the neurological disease or disorder is selected from Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury and glaucoma.

[0034] Further provided is use of the multispecific antibody of the present disclosure in the manufacture of an agent or a kit for diagnosing Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury or glaucoma.

[0035] Further provided is use of the multispecific antibody of the present disclosure in the manufacture of a medicament for inhibiting and/or decreasing the formation of amyloid plaques in the brain.

[0036] In yet another aspect, provided is a method of transporting a compound across the blood-brain barrier in a subject, comprising conjugating the anti-TFR1 antibody or antigen-binding fragment of the present disclosure to the compound and transporting the conjugate across the blood-brain barrier.

[0037] In a further aspect, provided is a method of treating a neurological disease or disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the anti-TFR1 antibody or antigen-binding fragment of the present disclosure conjugated to a therapeutic agent for treating the neurological disease or disorder to the subject.

[0038] In a particular aspect, provided is a method of treating a neurological disease or disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the multispecific antibody of the present disclosure to the subject. In some embodiments, the neurological disease or disorder is selected from Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury and glaucoma.

[0039] Further provided is a method of inhibiting and/or decreasing the formation of amyloid plaques in the brain of a subject in need thereof, comprising administering a therapeutically effective amount of the multispecific antibody of the present disclosure to the subject to inhibit and/or decrease the formation of amyloid plaques in the brain.

[0040] In another aspect, provided is the anti-TFR1 antibody or antigen-binding fragment of the present disclosure for use as a medicament, wherein the anti-TFR1 antibody or antigen-binding fragment is transporting a compound across the blood-brain barrier.

[0041] In yet another aspect, provided is the anti-TFR1 antibody or antigen-binding fragment of the present disclosure for use in treating cancer.

[0042] In a further aspect, provided is the anti-TFR1 antibody or antigen-binding fragment of the present disclosure for use as a medicament for treating a neurological disease or disorder, wherein the anti-TFR1 antibody or antigen-binding fragment is conjugated to a therapeutic agent for treating the neurological disease or disorder.

[0043] In a particular aspect, provided is the multispecific antibody of the present disclosure for use in treating a neurological disease or disorder, optionally, wherein the neurological disease or disorder is selected from Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury and glaucoma.

[0044] Further provided is the multispecific antibody of the present disclosure for use in diagnosing Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury or glaucoma.

[0045] Also provided is the multispecific antibody of the present disclosure for use in inhibiting and/or decreasing the formation of amyloid plaques in the brain.

Brief Description of the Drawings

[0046] Figure 1 illustrates the binding affinity of anti-TFR1 HCAb antibodies to human TFR1-His protein.

[0047] Figure 2 illustrates the binding affinity of anti-TFR1 HCAb antibodies to cyno TFR1-His protein.

[0048] Figure 3 illustrates the binding affinity of anti-TFR1 HCAb antibodies to HEK293T-huTFR1 cells.

[0049] Figure 4 illustrates the binding affinity of anti-TFR1 HCAb antibodies to HEK293T-cyno TFR1 cells.

[0050] Figure 5 illustrates the binding affinity of anti-TFR1 HCAb antibodies to CHOK1-hCD40 cells.

[0051] Figure 6 illustrates the epitope binning of anti-TFR1 HCAb antibodies by competition assay.

[0052] Figure 7 illustrates the blocking effect of anti-TFR1 antibodies on the binding of human TF protein to human TFR1 by ELISA.

[0053] Figure 8 illustrates the activity of anti-TFR1 antibodies in blocking the binding of human TF to TFR1 by FACS.

[0054] Figure 9 illustrates the binding affinity of PR012592 mutants to human TFR1-His protein.

[0055] Figure 10 illustrates the binding affinity of PR012592 mutants to cyno TFR1-His protein.

[0056] Figure 11 illustrates the binding affinity of PR012592 mutants to HEK293T-huTFR1 cells.

[0057] Figure 12 illustrates the schematic diagram of BACE1×TFR1 BsAbs structure.

[0058] Figure 13 illustrates the schematic diagram of Aβ×TFR1 BsAbs structure.

[0059] Figure 14 illustrates binding affinity of Aβ×TFR1 BsAbs to human TFR1-His protein.

[0060] Figure 15 illustrates binding affinity of BACE1×TFR1 BsAbs to human TFR1-His protein.

[0061] Figure 16 illustrates binding affinity of Aβ×TFR1 BsAbs to HEK293T-huTFR1 cells.

[0062] Figure 17 illustrates binding affinity of BACE1×TFR1 BsAbs to HEK293T-huTFR1 cells.

[0063] Figure 18 illustrates binding affinity of BACE1×TFR1 BsAbs to CHOK1-cyno TFR1 cells.

[0064] Figure 19 illustrates the schematic diagram of *in vivo* study design.

[0065] Figure 20 illustrates the relative drug concentration in brain parenchyma.

[0066] Figure 21 illustrates the relative drug concentration in whole brain.

[0067] Figure 22 illustrates the relative drug concentration in serum.

[0068] Figure 23 illustrates the IHC staining for brain cortex.

Detailed Description**Definitions**

[0069] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art. In addition, the terms and experimental procedures relating to protein and nucleic acid chemistry, molecular biology, cell and tissue culture, microbiology and immunology are those terms and common procedures widely used in the art. Meanwhile, for better understanding of the present disclosure, definitions and explanations of relevant terms are provided below.

[0070] As used herein, the expression “at least one” or “one or more” refers to one, two, three, four, five, six or more. As used herein, “a” and “an” unless clearly indicated to the contrary, should be understood to mean “at least one”.

[0071] As used herein, the term “antigen (Ag)” is any substance which could trigger immune response in the body, especially the production of antibodies. As used herein, this term refers to any substance comprising epitope(s) (i.e., antigenic determinant(s)) to which an antibody specifically binds. An antigen can be a polypeptide, lipid, carbohydrate, polynucleotide, etc. In a specific embodiment, the antigen refers to TFR1 proteins or fragments thereof comprising the epitope to which the antibody or antigen-binding fragment of the present disclosure binds. The TFR1 proteins or fragments thereof may be of any species, such as human, murine and rabbit or the like.

[0072] As used herein, the term “antibody (Ab)” is an immunoglobulin (Ig) molecule or fragment thereof that specifically binds to the epitope of an antigen through at least one antigen-binding site (i.e., the paratope). Antibodies may be derived from different species, including but not limited to mouse, rat, rabbit, guinea pig and human.

[0073] As used herein, the definition of “antibody” encompasses conventional antibodies, single domain antibodies, heavy-chain-only antibodies, recombinant antibodies, multispecific antibodies (e.g., bispecific antibodies), monoclonal antibodies, polyclonal antibodies, fully human antibodies, non-human antibodies, humanized antibodies, chimeric antibodies, intrabodies, diabodies, anti-idiotypic antibodies, and antigen-binding fragments. Antibodies provided herein include members of any immunoglobulin type (e.g., IgG, IgM, IgD, IgE, IgA and IgY), any class (e.g. IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass (e.g., IgG2a and IgG2b).

[0074] As used herein, the term “heavy-chain-only antibody” or “heavy-chain antibody” or “HCAb” refers to an antibody consisting only of two heavy chains and lacking the two light chains usually found in conventional antibodies. In contrast to a conventional antibody, an HCAb only require the heavy chain variable domain (also referred to as “VHH”) for antigen binding. That is, the antigen-binding site of an HCAb is primarily determined by the three CDRs of the VHH. Typically, the heavy chains of HCABs lack the CH1 domain usually found in the heavy chains of conventional antibodies. HCABs can be obtained by hybridoma technology, or phage libraries or eukaryotic cell libraries displaying or secreting HCABs.

HCAbs can also be obtained from transgenic animals, for example transgenic mice capable of producing fully human HCAbs (see e.g., WO2007/096779).

[0075] As used herein, the term “single-domain antibody” or “nanobody” refers to an antibody only consists of a single variable domain. The single variable domain may be a heavy chain single variable domain or a light chain single variable domain.

[0076] A “conventional” or “full-length” antibody typically consists of four polypeptides: two heavy chains (HC) and two light chains (LC). Each light chain, from the amino-terminus to the carboxyl-terminus, comprises a light chain variable region (VL) and a light chain constant region (CL). Each heavy chain, from the amino-terminus to the carboxyl-terminus, comprises a heavy chain variable region (VH) and one or more heavy chain constant regions (CH), which could include CH1, CH2, CH3 and CH4.

[0077] The term “monoclonal antibody” as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding specificity and affinity. In one embodiment, the monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a non-human animal, e.g., mouse, fused to an immortalized cell.

[0078] The term “recombinant antibody”, as used herein, includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal with respect to the immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences.

[0079] The term “human antibody”, as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. Human antibodies may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*). The definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0080] The term “chimeric antibody” refers to those antibodies wherein one portion of each of the amino acid sequences of heavy and light chains is homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular class, while the remaining segment of the chain is homologous to corresponding sequences in another.

[0081] As used herein, a “humanized antibody” is an antibody derived from a non-human antibody whose sequence has been modified to increase the sequence similarity to human immunoglobulin molecules.

[0082] As used herein, the term “fully human antibody” refers to an antibody that comprises human immunoglobulin protein sequences only. A fully human antibody may contain murine

carbohydrate chains if produced in a mouse, in a mouse cell, or in a hybridoma derived from a mouse cell.

[0083] As used herein, an “antigen-binding fragment” of an antibody refers to any portion of a full-length antibody that contains at least a portion of the variable domains (e.g. one or more CDRs) of the antibody and specifically binds to the same cognate antigen as the full-length antibody. Examples of antigen-binding fragments include, but are not limited to, Fv, scFv, dsFv, scdsFv, diabody, single variable domain (e.g., VHH), Fd, di-scFv, Fab, Fab', scFab, F(ab')₂, and other fragments. An antigen-binding fragment generally contains at least or about 50 amino acids and typically at least or about 200 amino acids.

[0084] A single-chain variable fragment (scFv) is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a peptide linker. The peptide linker can either connect the N-terminus of the VH with the C-terminus of the VL, or vice versa.

[0085] The Fv or the scFv can be further stabilized by introducing an interchain disulfide bond between the VL and the VH to generate a disulfide-stabilized variable fragment (dsFv) or a single-chain disulfide-stabilized variable fragment (scdsFv, with a peptide linker and an interchain disulfide bond), respectively.

[0086] Two single-chain variable fragments may be linked by a peptide linker to generate a covalently linked divalent single chain (referred to as an “sc(Fv)₂”). A “diabody” is a noncovalent dimer of scFv.

[0087] The term “di-scFv” refers to a fusion protein comprising two light chain variable region (VL) and two heavy chain variable region (VH), wherein the two VL and the two VH are contiguously linked optionally via polypeptide linkers, and capable of being expressed as a single chain polypeptide. The two VL and two VH are fused optionally by peptide linkers to form a bivalent molecule, such as in the order of VL₁-linker-VH₁-linker-VL₂-linker-VH₂ or VL₁-linker-VH₁-linker-VH₂-linker-VL₂ or VL₁-linker-VL₂-linker-VH₂-linker-VH₁ or VL₁-linker-VH₂-linker-VL₂-linker-VH₁, to form two antigen-binding domains.

[0088] An Fab fragment essentially comprises a light chain and one variable and one constant region of the heavy chain (i.e., the VH-CH₁, also known as the fragment difficult (Fd)), wherein the VL and VH form the antigen-binding site (also known as the fragment variable region, Fv), and the CL and CH₁ are covalently linked by a disulfide bond.

[0089] A single-chain Fab (scFab) fragment may be generated by connecting the light chain and the Fd fragment with a peptide linker.

[0090] An F(ab')₂ fragment essentially contains two Fab fragments joined at the hinge region through disulfide bonds. An Fab' fragment is one half of a F(ab')₂ fragment, formed by the reduction of the disulfide bonds at the hinge region of the F(ab')₂ fragment.

[0091] As used herein, the term “Fc region” refers to a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the heavy chain constant region. Preferably, an Fc region comprises at least a CH₃ domain, for example, a CH₃ domain, a CH₂

domain and a CH3 domain or preferably, a hinge region, a CH2 domain and a CH3 domain. This term encompasses native and variant Fc regions. Unless otherwise specified, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system (EU index), as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[0092] In some embodiments, an antibody or antigen-binding fragment as described herein comprises an Fc region. In some embodiments, the Fc region comprises a CH3 domain. In some embodiments, the Fc region comprises a CH2 domain and a CH3 domain. In some embodiments, the Fc region comprises at least a portion of a hinge region, a CH2 domain and a CH3 domain. In some embodiments, for example, wherein the Fc region is fused to the C-terminus of the heavy chain variable domain binding to TFR1 of the present disclosure, the Fc region lacks a CH1 domain. The Fc region may be a monomeric Fc region, i.e., a soluble monomeric Fc region (see e.g., Wang C et al., Engineered Soluble Monomeric IgG1 Fc with Significantly Decreased Non-Specific Binding. Front Immunol. 2017 Nov 13;8:1545), or a dimeric Fc region, which comprises two identical (homodimer) or different (heterodimer) Fc region subunits.

[0093] The Fc region may be used may be derived from any immunoglobulin subtype or subclass, for example, IgG, IgM, IgA, IgD and IgE. Preferably, the Fc region is derived from human IgG, e.g., human IgG1, IgG2, IgG3 and IgG4. In some embodiments, the Fc region is a human IgG1 Fc region. In some embodiments, a human IgG1 Fc region may extend from Glu216, or Cys220, or Asp221, or Cys226, or Pro230, to the carboxyl-terminus of the heavy chain, while the C-terminal lysine (Lys447) of the heavy chain may or may not be present. In some embodiments, a heavy chain variable domain is fused to the C-terminal of the Fc region, the C-terminal Lys447 of the heavy chain may be deleted.

[0094] The Fc regions of antibodies may be modified to obtain antibodies having desirable properties. For example, one or more cysteines may be introduced into or removed from the hinge region (e.g., by site-directed mutagenesis) to promote or weaken dimerization. For example, hinge region, CH2 and/or CH3 may be modified to prolong or decrease the half-life, promote the cellular internalization or tissue penetration, increase or decrease the binding to FcγRIIB (see e.g., WO2008150494A1), increase or decrease Fc effector functions, including but not limited to antibody-dependent cell-mediated cytotoxicity (ADCC), antibody dependent cell-mediated phagocytosis and complement-dependent cytotoxicity (CDC) (see e.g., Caron, P. C., et al., J. Exp. Med. 176:1191-1195 (1992); and Shopes B.J. Immunol.148 2918-2922(1992)).

[0095] The term “hinge region” generally refers to a portion between the CH1 and CH2 domains of a conventional antibody, a portion between the VHH and CH2 domains of a heavy chain antibody or a functional equivalent thereof (e.g., the hinge region in a T cell receptor (TCR)). The hinge region may be an intact hinge region or a portion thereof.

[0096] The variable region (or “variable domain”) allows specific antigen recognition and binding. Each variable domain has three hypervariable “complementarity-determining regions

(CDRs)” and four relatively conserved “framework regions (FWRs)”, arranged from the amino-terminus (N-terminus) to the carboxyl-terminus (C-terminus) in the following order: FWR1, CDR1, FWR2, CDR2, FWR3, CDR3, FWR4. One of skill in the art knows and can identify the CDRs and FWRs using methodology of the art, for example, the Kabat or Chothia numbering schemes (see e.g., Kabat, E.A. *et al.* (1991) *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. *et al.* (1987) *J. Mol. Biol.* 196:901-917).

[0097] Each CDR forms a CDR loop and contributes to the antigen-binding specificity and affinity. The framework residues form a scaffold and maintain the overall structure of the antigen-binding domain (e.g., Fv or VHH). The combinations of CDR loops (e.g., three CDRs from each of VL and VH, or the three CDRs from VHH), with the help of the FWRs, form a three-dimensional structure, which defines the paratope or the antigen-binding site of the antibody. According to the locations in the VL or VH (or VHH), the CDR is designated as the heavy chain variable region CDR (HCDR or VH CDR), like HCDR1 (or VH CDR1), HCDR2 (or VH CDR2), HCDR3 (or VH CDR3); or the light chain variable region CDR (LCDR or VL CDR), like LCDR1 (or VL CDR1), LCDR2 (or VL CDR2), LCDR3 (or VL CDR3).

[0098] In a given amino acid sequence of an immunoglobulin variable region, the precise amino acid sequence boundary of each CDR may be determined by using any one of many well-known antibody numbering schemes or a combination thereof, wherein the numbering schemes include, for example, Chothia based on the three-dimensional structures of antibodies and the topology of CDR loops (Chothia *et al.*, (1989) *Nature* 342: 877-883, Al-Lazikani *et al.*, “Standard Conformations for the Canonical Structures of Immunoglobulins”, *Journal of Molecular Biology*, 273, 927-948 (1997)), Kabat based on the antibody sequence variability (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, version 4, U.S. Department of Health and Human Services, National Institutes of Health (1987)), AbM (University of Bath), Contact (University College London), the International ImMunoGeneTics database (IMGT) (World-Wide-Web imgt.cines.fr/), and the North CDR definition based on affinity propagation clustering using a large number of crystal structures. In the anti-TFR1 antibody or antigen-binding fragment of the present disclosure, the amino acid sequences of the CDR regions are defined using the Chothia numbering scheme, and it is not meant that the present disclosure only protects an antibody molecule represented by the CDR regions defined by the Chothia numbering scheme. It shall be noted that the boundaries of CDRs of a variable region in the same antibody obtained based on different numbering scheme may be different, that is, the CDR sequences of the variable region in the same antibody defined under different numbering schemes may be different. Once a variable region (e.g., a VH or VL) is given, those skilled in the art would understand that CDRs within the variable region can be defined by different numbering systems or combinations thereof. The CDR sequences defined under these different numbering schemes and the antibody molecules characterized thereby are also intended to be protected by the present disclosure.

[0099] Those skilled in the art would be able to determine the positions of the CDRs, FWRs, VHH, VH, CH1, hinge region, CH2, CH3, VL and CL in an antibody, for example with the help of an algorithm or a software (see e.g., William R. Strohl, Lila M. Strohl, (2012), Antibody structure–function relationships, In Woodhead Publishing Series in Biomedicine, Therapeutic Antibody Engineering, Woodhead Publishing, pp. 37-56).

[0100] The term “multispecific antibody” is used in the broadest sense and specifically covers an antibody that is capable of specifically binding to two or more different epitopes. The two or more different epitopes may be present on the same biological molecule or on two or more different biological molecules. A multispecific antibody may be a multivalent (e.g., bivalent, trivalent, tetravalent, pentavalent or more) antibody. A multispecific antibody may be a bispecific, trispecific or tetraspecific antibody, which can specifically bind to 2, 3 or 4 different epitopes. Exemplary multispecific antibodies may bind both TFR1 and a brain antigen. In some embodiments, the multispecific antibody is a bispecific antibody. In some embodiments, the multispecific antibody binds TFR1 and BACE1 (“anti-TFR1/BACE1 antibody”) or TFR1 and A β (“anti-TFR1/A β antibody”).

[0101] Multispecific antibodies include, but are not limited to, full length antibodies, antibody fragments such as Fab, Fv, dsFv, scFv, diabodies, single-chain diabodies, bispecific diabodies and triabodies, antibody fragments that have been linked covalently or non-covalently. A multispecific antibody may be a non-IgG like antibody or an IgG-like antibody. An IgG-like multispecific antibody may be symmetric or asymmetric. Methods for constructing multispecific antibodies using antibodies or antigen-binding fragments of interest are well known to those skilled in the art (see e.g., WO93/08829; Suresh et al., (1986) Methods in Enzymology, 121: 210; and Traunecker et al., (1991) EMBO, 10: 3655-3659). Multispecific antibodies may be generated and isolated using various methods known in the art. For example, a multispecific antibody may be expressed and isolated from a suitable host or generated by conjugating different portions of the multispecific antibody by chemical or enzymatic methods.

[0102] As used herein, a “bispecific antibody” is a multispecific antibody that is capable of specifically binding to two different epitopes. The two different epitopes may be present on the same biological molecule or on two different biological molecules.

[0103] The term “knob-into-hole” or “KiH” technology as used herein refers to the technology directing the pairing of two polypeptides together *in vitro* or *in vivo* by introducing a protuberance (knob) into one polypeptide and a cavity (hole) into the other polypeptide at an interface in which they interact. For example, KiHs have been introduced in the Fc:Fc binding interfaces, CL:CH1 interfaces or VH/VL interfaces of antibodies (see, e.g., US2011/0287009, US2007/0178552, WO96/027011, WO98/050431, and Zhu et al., 1997, Protein Science 6:781-788). In some embodiments, KiHs drive the pairing of two different heavy chains together during the manufacture of multispecific antibodies.

[0104] As used herein, the term “affinity” is a measurement of the strength of the noncovalent interaction between an antibody and its antigen.

[0105] Various methods known in the art may be used to measure the affinity. Such methods may include, but are not limited to, Bio-Layer Interferometry (BLI, e.g., using the Octet ForteBio system), radioimmunoassay (RIA), Surface Plasmon Resonance (SPR), enzyme linked immunosorbent assay (ELISA), flow cytometry (also referred to as “FACS”) and other kinetic interaction assays known in the art (see, e.g., Paul, W. E., *Fundamental Immunology*, 2nd ed., Raven Press, New York, pages 332-336 (1989); see also U.S. Pat. No. US7,229,619). Instrumentation and methods for real time detection and monitoring of binding rates are known and are commercially available (e.g., BiaCore 2000, Biacore AB, Upsala, Sweden).

[0106] Typically, the antibody affinity may be measured and reported by the equilibrium dissociation constant K_D . K_D can be calculated from kinetic analysis using the following equation:

$$K_D = K_d / K_a$$

wherein K_d and K_a are the dissociation and association rate constants between the antigen and the antibody, respectively.

[0107] The K_D and affinity are inversely related, that is, the smaller the K_D value, the greater the affinity. The typical K_D value for an antibody or antigen-binding fragment is in the range of μM (10^{-6} M) to nM (10^{-7} to 10^{-9} M). In some embodiments, the K_D values described herein are measured using Bio-Layer Interferometry as demonstrated in the Examples of the present disclosure.

[0108] As used herein, the term “half maximal effective concentration” or “EC₅₀” refers to the concentration of an agent (e.g., antibody) at which it induces a response halfway between the baseline and maximum after some specified exposure time. EC₅₀ can be used as a surrogate measurement for the affinity of an antibody for its target.

[0109] As used herein, “specifically bind” refers to the affinity between an antibody or antigen-binding fragment and its cognate antigen. Generally, an antibody specifically binds to its cognate antigen with a high affinity, or with a K_D value of 10^{-7} to 10^{-9} M or less, preferably with a K_D value of 10^{-8} or less, 10^{-9} or less, 10^{-10} or less, 10^{-11} or less, or 10^{-12} or less. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment specifically bind to human TFR1.

[0110] The definition of “antibody” or “antigen-binding fragment” encompasses any variant derived from any antibody or antigen-binding fragment as described herein, such as amino acid sequence variants, glycosylation variants, and covalently modified variants. The definition of antigen-binding protein falls within the definition of an antibody or antigen-binding fragment.

[0111] An “amino acid sequence variant” may comprise one or more amino acid modifications (including substitutions, insertions and/or deletions) compared to an exemplary antibody as described herein, provided that the amino acid sequence variant preserves the desired antigen-binding activity. For example, an amino acid sequence variant may be obtained by substituting one or more amino acid residues with corresponding conservative residues without affecting the biological activity of the protein. Suitable conservative substitutions of

amino acids are known to those of skill in this art. Those of skill in this art recognize that, in general, single amino acid substitutions in non-essential regions of a polypeptide (for example, in the framework regions of an antibody) do not substantially alter its biological activity (see, e.g., Watson et al., *Molecular Biology of the Gene*, 4th Edition, 1987, The Benjamin/Cummings Pub. Co., p.224).

[0112] Amino acid sequence variants also include “Fc variants” of any antibody or antigen-binding fragment as described herein. Such Fc variants may comprise one or more modifications in the Fc region, such that one or more properties of the Fc region may be changed. For example, modifications may be introduced into the Fc region to increase or reduce the effector function (including but not limited to ADCC, CDC and ADCP), increase the half-life (for example, enhanced FcRn binding) or increase the stability (for example, introduction or removal of cysteines that may be involved in disulfide-bond formation) of an antibody.

[0113] In some embodiments, for example, which relate to treatment of a neurological disease or disorder, reduced effector function of the anti-TFR1 antibody, the TFR1-binding agent, or the multispecific antibody of the present disclosure may be desirable. One or more modifications for reducing the effector function may include but not limited to one or more modifications (e.g., amino acid substitutions) at a position in the Fc region selected from: 297 (e.g., N297A, N297Q or N297G), 234 (IgG1: L234A; IgG4: F234A; IgG2: V234A), 235 (e.g., L235E, L235A), 237 (G237A).

[0114] One or more modifications for enhancing the FcRn binding may include but not limited to one or more modifications (e.g., amino acid substitutions) at a position in the Fc region selected from: 252 (e.g., M252Y), 254 (e.g., S254T), 256 (e.g., T256E), 428 (e.g., M428L), 434 (e.g., N434S and N434A) and 436 (e.g., Y436I). In some embodiments, the Fc region comprises modifications of M252Y, S254T and T256E. In some embodiments, the Fc region comprises modifications of N434A and Y436I.

[0115] In some embodiments, the Fc region comprises a modification of C220S. In some embodiments, the Fc region comprises modifications of L234A, L235A and G237A. In some embodiments, the Fc region is a human IgG1 Fc region comprising one or more modifications selected from: C220S, L234A, L235A and G237A. In some embodiments, the human IgG1 Fc region comprises a modification of C220S. In some embodiments, the human IgG1 Fc region comprises modifications of L234A, L235A and G237A. In some embodiments, the human IgG1 Fc region comprises modifications of C220S, L234A, L235A and G237A.

[0116] As used herein, “glycosylation” refers to a post-translational modification of a protein leading to the addition of carbohydrate or glycan moieties to the protein backbone (e.g., serine- or threonine-linked glycosylation for O-linked glycans and asparagine-linked glycosylation for N-linked glycans). Those of skill in the art are able to generate glycosylation variants of a therapeutic antibody to achieve desired therapeutic efficacies using techniques known in the art.

[0117] Also contemplated are covalently modified variants of any antibody or antigen-binding fragment as described herein. As used herein, “covalently modified variants” of an antibody or antigen-binding fragment can be generated by introducing natural or unnatural amino acids, compounds, or peptide linkers through covalently linkage. For example, an additional polypeptide may be linked to an antibody or antigen-binding fragment as described herein to confer a desirable property or reduce an undesirable property, for example increasing the half-life, solubility or uptake (e.g., facilitating the transport through the phospholipid membrane or organelles); and/or lowering the immunogenicity, toxicity or side effects. Other polypeptides may be, for example, polypeptides that can be used for directing the expression and secretion of an antibody or antigen-binding fragment from a host cell, or facilitating the detection and/or isolation of an antibody or antigen-binding fragment. Examples of such polypeptides may include but are not limited to signaling peptides (leader sequences), affinity tags (e.g., polyhistidine tags (His₆) or glutathione S-transferase (GST) tags), polypeptides containing protease cleavage sites and reporter tags (e.g., fluorescent proteins). Other additional polypeptides may be biologically active polypeptides, for example, a polypeptide or protein having a therapeutic activity, binding activity or enzymatic activity.

[0118] As used herein, the term “transferrin receptor 1” or “TFR1” refers to any native TFR1 from any vertebrate source, including mammals such as primates (e.g., human and cynomolgus) and rodents (e.g., mice and rats). This term encompasses “full-length” TFR1 as well as a fragment of TFR1. The term also encompasses naturally occurring variants of TFR1, e.g., splice variants or allelic variants. In some embodiments, the TFR1 is human TFR1 (“hTFR1” or “huTFR1”), for example, a human TFR1 comprising the amino acid sequence as set forth in Uniprot accession No.: P02786. In another embodiment, the TFR1 is cynomolgus TFR1 (“cynoTFR1”), for example a cynomolgus TFR1 comprising the amino acid sequence as set forth in Uniprot accession No.: G8F602.

[0119] As used herein, the term “blood-brain barrier” or “BBB” refers to the physiological barrier between the peripheral circulation and the brain and spinal cord (i.e., the CNS) which is formed by tight junctions within the brain capillary endothelial plasma membranes, creating a tight barrier that restricts the transport of molecules into the brain, even very small molecules such as urea (60 Daltons). The blood-brain barrier within the brain, the blood-spinal cord barrier within the spinal cord, and the blood-retinal barrier within the retina are contiguous capillary barriers within the CNS and are herein collectively referred to the BBB. The definition of BBB also encompasses the blood-cerebrospinal fluid (CSF) barrier where the barrier is comprised of ependymal cells rather than capillary endothelial cells.

[0120] As used herein, a “brain antigen” refers to an antigen (or target) expressed in the CNS, including the brain, which can be targeted with an antibody or small molecule. Examples of brain antigens may include, but are not limited to: beta-secretase 1 (BACE1), amyloid precursor protein (APP), amyloid beta (A β or A β), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E4 (ApoE4), alpha-

synuclein, CD20, huntingtin, prion protein, leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), p75 neurotrophin receptor (p75NTR), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1 β), and caspase 6.

[0121] The term “BACE1” as used herein, refers to any native beta-secretase 1 from any vertebrate, including mammals such as primates (e.g. humans) and rodents (e.g., mice and rats), unless otherwise indicated. This term encompasses “full-length” unprocessed BACE1 as well as any form of BACE1 which results from processing in the cell. This term also encompasses naturally occurring variants of BACE1, e.g., splice variants or allelic variants.

[0122] The term “amyloid precursor protein” or “APP” as used herein refers to a protein that can be proteolytically processed or cleaved by one or more processing or cleavage reactions to produce A β . APP includes all isoforms that are generated by alternative splicing.

[0123] The terms “amyloid beta” and “Abeta (A β)” are used interchangeably to refer to the fragment of amyloid precursor protein (“APP”) that is produced upon BACE1 cleavage of APP, as well as modifications, fragments and any functional equivalents thereof, including, but not limited to, A β 1-40, and A β 1-42.

[0124] The term “amyloid plaque” as used herein refers to an insoluble fibrous protein aggregate including amyloid beta. Amyloid plaques may be found in the CNS (e.g., brain), for example, around dystrophic neurites, axonal terminals and dendrites, microglia and fibrous astrocytes.

[0125] As used herein, a “neurological disease or disorder” refers to a disease or disorder which affects the central nervous system (CNS) and/or which has an etiology in the CNS. Exemplary neurological diseases or disorders include, but are not limited to, neuropathy, amyloidosis, brain cancer, an ocular disease or disorder, viral or microbial infection, inflammation, ischemia, neurodegenerative disease, seizure, behavioral disorders, and a lysosomal storage disease.

[0126] Examples of neurological diseases or disorders may include, but are not limited to: neurodegenerative diseases (e.g., Lewy body disease, postpoliomyelitis syndrome, Shy-Draeger syndrome, olivopontocerebellar atrophy, Parkinson’s disease, multiple system atrophy), striatonigral degeneration, tauopathies (e.g., Alzheimer’s disease and supranuclear palsy), prion diseases (e.g., bovine spongiform encephalopathy, scrapie, Creutzfeldt-Jakob syndrome, kuru, Gerstmann-Straussler-Scheinker disease, chronic wasting disease, and fatal familial insomnia), bulbar palsy, motor neuron disease, and nervous system heterodegenerative disorders (e.g., Canavan disease, Huntington’s disease, neuronal ceroid-lipofuscinosis, Alexander disease, Tourette syndrome, Menkes kinky hair syndrome, Cockayne syndrome, Halervorden-Spatz syndrome, lafora disease, Rett syndrome, hepatolenticular degeneration, Lesch-Nyhan syndrome, and Unverricht-Lundborg syndrome), dementia (e.g., Pick’s disease, and spinocerebellar ataxia), central nervous system cancer (including primary brain tumors and brain metastases resulting from cancer elsewhere in the body).

[0127] As used herein, the term “polypeptide” refers to two or more amino acids covalently joined. The terms “polypeptide” and “protein” are used interchangeably herein.

[0128] As used herein, the term “first polypeptide (chain)” and similar terms like “second polypeptide (chain)”, “third polypeptide (chain)” and “fourth polypeptide (chain)” are used for the purpose of distinguishing only, but not intended to constrain the polypeptides (chains) in any manner. If desired, an antibody or antigen-binding fragment may comprise two or more first/second/third/fourth polypeptides (chains) which may be identical or different. When used in different embodiments, these terms may refer to identical or different polypeptides (chains).

[0129] As used herein, the term “cancer” refers to proliferative diseases. This term encompasses solid cancers and hematopoietic malignancies. Examples of solid cancers may include but are not limited to esophageal squamous cell carcinoma, breast cancer, ovarian cancer, lung cancer, cervical cancer, bladder cancer, osteosarcoma, pancreatic cancers, bile duct cancer, renal cell carcinoma, hepatocellular carcinoma, adrenal cortical carcinoma, and cancers of the nervous system. Examples of hematopoietic malignancies may include but are not limited to Hodgkin’s lymphoma, non-Hodgkin’s lymphoma, leukemia (e.g., acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myelogenous leukemia, chronic lymphoblastic leukemia, and chronic lymphoid leukemia) and multiple myeloma.

[0130] As used herein, a “nucleic acid” or a “polynucleotide” refers to a polymer of at least two nucleotides or nucleotide derivatives joined together by phosphodiester bonds, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Exogenous nucleic acids can be introduced into a host cell in the form of vectors. Therapeutic nucleic acids can be introduced into a subject with a purpose of treating a disease.

[0131] A “host cell” may be any prokaryotic or eukaryotic cell that contains exogenous polynucleotides.

[0132] As used herein, the term “expression” refers to the production of RNA polynucleotides from transcription or polypeptides from translation. The expression level of a polypeptide in a biological sample can be examined using any method known in art. Such methods include, but are not limited to, enzyme-linked immunosorbent assay (ELISA), western blot, flow cytometry, immunofluorescence imaging and immunohistochemistry using antibodies that specifically bind to the polypeptide to be examined.

[0133] As used herein, a “vector” is a vehicle used to transfer exogenous nucleic acids into a host cell, where the exogenous nucleic acids are amplified or expressed. As used herein, the definition of “vector” encompasses plasmids, linearized plasmids, viral vectors, cosmids, phage vectors, phagemids, artificial chromosomes (e.g., yeast artificial chromosomes and mammalian artificial chromosomes), etc. A vector could be expressible and/or replicable inside a host cell, meaning that the vector is able to express RNA polynucleotides or polypeptides and/or to produce multiple copies of the vector in the host cell. To be “expressible” or “replicable”, a vector could comprise nucleic acid sequences or elements operably linked to a promoter.

[0134] As used herein, “operably linked” with reference to nucleic acid sequences or elements means that these nucleic acid sequences are functionally related to each other. For example, a promoter can be operably linked to a nucleic acid sequence encoding a polypeptide, whereby the promoter regulates or mediates the transcription of the nucleic acid. Those skilled in the art could select and use appropriate vectors for a particular purpose.

[0135] As used herein, “treating” a disease or disorder refers to curing the disease or disorder, or arresting, alleviating, ameliorating or eliminating the symptoms of the disease or disorder. Hence treatment encompasses prophylaxis, therapy and/or cure. “Prophylaxis” refers to prevention of a potential disease and/or a prevention of worsening of symptoms or progression of a disease.

[0136] As used herein, a “therapeutically effective amount” refers to the quantity of an agent, compound, or composition containing one or more active agents that is at least sufficient to produce a therapeutic effect following administration to a subject. Hence, it is the quantity necessary for preventing, curing, ameliorating, arresting or partially arresting a symptom of a disease or disorder.

[0137] As used herein, the term “subject” refers to an animal (e.g., a mammal), including, but not limited to, humans, non-human primates, canines, felines, rabbits, rodents, and the like. In some embodiments, the subject is a human.

[0138] The antibody identity numbers or identifies (e.g., PR012592, PR012838, PR012839, PR013071, PR013072, PR013356, PR013357, PR013358, PR013359, PR013360, PR012932, PR013352, PR013353, PR013354, PR013355 or the like) are used herein for the purpose of distinguishing or designating different antibodies or products only, but do not intend to restrict the features of the antibody or product of the present disclosure thereto. Similarly, the use of identity numbers or identifies in the Examples is merely for convenience and brevity. The features of the antibody or product of the present disclosure are defined by those described in the appended claims.

Anti-TFR1 antibodies or antigen-binding fragments thereof

[0139] In one aspect, provided is an anti-transferrin receptor 1 (TFR1) antibody or an antigen-binding fragment thereof.

[0140] In some embodiment, the anti-TFR1 antibody or antigen-binding fragment binds to human TFR1 and non-human primate (e.g., cynomolgus monkey) TFR1. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment does not substantially affect the binding of transferrin to TFR1. The term “not substantially affecting” includes not inhibiting/decreasing and not increasing the binding of transferrin to TFR1. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment binds to the transferrin-TFR1 complex.

[0141] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment comprises a heavy chain variable domain. In some embodiments, the heavy chain variable domain is a heavy chain single variable domain.

[0142] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment is a single domain antibody. In some particular embodiments, the anti-TFR1 antibody or antigen-binding fragment is a heavy-chain-only antibody.

[0143] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment is a fully human antibody. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment is a fully human single domain antibody. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment is a fully human heavy-chain-only antibody.

[0144] In some embodiments, the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3.

[0145] In some embodiments, the VH CDR1 comprises an amino acid sequence differing from SEQ ID NO: 8 by amino acid addition, deletion or substitution of no more than 2 amino acids.

[0146] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8.

[0147] In some embodiments, the VH CDR2 comprises an amino acid sequence differing from SEQ ID NO: 16 by amino acid addition, deletion or substitution of no more than 2 amino acids.

[0148] In some embodiments, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16.

[0149] In some embodiments, the VH CDR3 comprises an amino acid sequence differing from SEQ ID NO: 24 by amino acid addition, deletion or substitution of no more than 2 amino acids.

[0150] In some embodiments, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 24.

[0151] In some embodiments, the VH CDR3 comprises a non-conservative amino acid substitution at a position corresponding to amino acid 2, 4, 5, or 7 of SEQ ID NO: 24. In some embodiments, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 79. In some embodiments, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 80. In some embodiments, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 81. In some embodiments, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 82.

[0152] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises an amino acid sequence differing from SEQ ID NO: 24 by amino acid addition, deletion or substitution of no more than 2 amino acids.

[0153] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises a non-conservative amino acid substitution at a position corresponding to amino acid 2, 4, 5, or 7 of SEQ ID NO: 24.

[0154] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 24, 79, 80, 81 or 82.

[0155] In some embodiments, the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in SEQ ID NO: 52. In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 24.

[0156] In some embodiments, the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 98. In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 79.

[0157] In some embodiments, the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 99. In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 80.

[0158] In some embodiments, the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 100. In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 81.

[0159] In some embodiments, the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 101. In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 82.

[0160] In some embodiments, the heavy chain variable domain further comprises a framework region 1 (FWR1), an FWR2, an FWR3 and an FWR4. The framework regions may be derived from an immunoglobulin of any species. In some preferable embodiments, the framework regions are derived from a human immunoglobulin.

[0161] In some embodiments, the FWR1 comprises the amino acid sequence of SEQ ID NO: 4. In some embodiments, the FWR2 comprises the amino acid sequence of SEQ ID NO: 12. In some embodiments, the FWR3 comprises the amino acid sequence of SEQ ID NO: 20. In some embodiments, the FWR4 comprises the amino acid sequence of SEQ ID NO: 27.

[0162] In some embodiments, the FWR1 comprises the amino acid sequence of SEQ ID NO: 4, and/or the FWR2 comprises the amino acid sequence of SEQ ID NO: 12, and/or the FWR3

comprises the amino acid sequence of SEQ ID NO: 20, and/or the FWR4 comprises the amino acid sequence of SEQ ID NO: 27.

[0163] In some embodiments, the FWR1 comprises the amino acid sequence of SEQ ID NO: 4, the FWR2 comprises the amino acid sequence of SEQ ID NO: 12, the FWR3 comprises the amino acid sequence of SEQ ID NO: 20, and the FWR4 comprises the amino acid sequence of SEQ ID NO: 27.

[0164] In some embodiments, the heavy chain variable domain comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101. In some of such embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 24, 79, 80, 81 or 82.

[0165] In some embodiments, the heavy chain variable domain comprises an amino acid sequence having amino acid addition, deletion and/or substitution of one or more (for example, 1-10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10) amino acids in comparison with the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101. In some preferable embodiments, the amino acid addition, deletion and/or substitution do/does not occur in a CDR region.

[0166] In some embodiments, the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO: 52. In some embodiments, the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO: 98. In some embodiments, the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO: 99. In some embodiments, the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO: 100. In some embodiments, the heavy chain variable domain comprises the amino acid sequence of SEQ ID NO: 101.

[0167] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment of the present disclosure further comprises an Fc region.

[0168] In some embodiments, the Fc region comprises one or more modifications to reduce the effector function. In some other embodiments, the Fc region comprises one or more modifications to enhance the effector function. In some embodiments, the effector function comprises at least one selected from ADCC, CDC and ADCP.

[0169] In some embodiments, the Fc region comprises one or more modifications to enhance the FcRn binding.

[0170] In some embodiments, the Fc region comprises one or more modifications to reduce the effector function and enhance the FcRn binding. In some embodiments, the Fc region comprises one or more modifications to enhance the effector function and enhance the FcRn binding.

[0171] In some embodiments, the Fc region is a monomeric Fc region. In some embodiments, the Fc region is a homodimeric Fc region. In some embodiments, the Fc region is a heterodimeric Fc region.

[0172] In some embodiments, the Fc region is directly fused to the C-terminus of the heavy chain variable domain. In some embodiments, the Fc region is fused to the N-terminus of the heavy chain variable domain, optionally via a peptide linker.

[0173] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment comprises a heavy chain which comprises a heavy chain variable domain as described herein fused to a monomeric Fc region.

[0174] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment comprises two heavy chains, wherein each heavy chain comprises a heavy chain variable domain as described herein fused to a Fc region subunit. In some embodiments, the two heavy chains are identical. In some embodiments, the two heavy chains are different.

[0175] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment comprises a heavy chain having the amino acid sequence of SEQ ID NO: 59. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment comprises a heavy chain having the amino acid sequence of SEQ ID NO: 107. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment comprises a heavy chain having the amino acid sequence of SEQ ID NO: 108. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment comprises a heavy chain having the amino acid sequence of SEQ ID NO: 109. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment comprises a heavy chain having the amino acid sequence of SEQ ID NO: 110.

[0176] In a particular aspect, the anti-TFR1 antibody or antigen-binding fragment is Antibody PR012592 as described herein. In some embodiments, Antibody PR012592 comprises a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 52, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 24. In some embodiments, Antibody PR012592 comprises a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 52. In some embodiments, Antibody PR012592 comprises a heavy chain having the amino acid sequence of SEQ ID NO: 59.

[0177] In a particular aspect, the anti-TFR1 antibody or antigen-binding fragment is Antibody PR012838 as described herein. In some embodiments, Antibody PR012838 comprises a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 98, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 79. In some embodiments, Antibody PR012838 comprises a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 98. In some embodiments, Antibody PR012838 comprises a heavy chain having the amino acid sequence of SEQ ID NO: 107.

[0178] In a particular aspect, the anti-TFR1 antibody or antigen-binding fragment is Antibody PR012839 as described herein. In some embodiments, Antibody PR012839 comprises a heavy

chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 99, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 80. In some embodiments, Antibody PR012839 comprises a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 99. In some embodiments, Antibody PR012839 comprises a heavy chain having the amino acid sequence of SEQ ID NO: 108.

[0179] In a particular aspect, the anti-TFR1 antibody or antigen-binding fragment is Antibody PR013071 as described herein. In some embodiments, Antibody PR013071 comprises a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 100, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 81. In some embodiments, Antibody PR013071 comprises a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 100. In some embodiments, Antibody PR013071 comprises a heavy chain having the amino acid sequence of SEQ ID NO: 109.

[0180] In a particular aspect, the anti-TFR1 antibody or antigen-binding fragment is Antibody PR013072 as described herein. In some embodiments, Antibody PR013072 comprises a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 101, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 82. In some embodiments, Antibody PR013072 comprises a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 101. In some embodiments, Antibody PR013072 comprises a heavy chain having the amino acid sequence of SEQ ID NO: 110.

[0181] In some aspects, provided are affinity modified variants of any antibody or antigen-binding fragment as described herein. An “affinity modified variant” may have increased or reduced antigen-binding affinity compared with any antibody or antigen-binding fragment as described herein but still specifically binds to TFR1 (e.g., hTFR1 or cynoTFR1). In some particular embodiments, the affinity modified variant has reduced antigen-binding affinity compared to Antibody PR012592.

[0182] In order to obtain an affinity modified variant, one or more amino acid modifications may be introduced to one or more CDR regions and/or framework regions of the antibody. Affinity modified variants may be obtained using methods known in the art, for example, by alanine-scanning mutagenesis in the CDR regions and/or framework regions and measuring the affinity of the alanine substitution variants to TFR1. Examples of such anti-TFR1 antibodies or antigen-binding fragments may include Antibodies PR012838, PR012839, PR013071 and PR013072 or an antigen-binding fragment thereof.

[0183] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment binds to human TFR1 and a non-human primate (for example, cynomolgus monkey) TFR1 with a K_D of about 5×10^{-8} M or less. In some preferable embodiments, the anti-TFR1 antibody or antigen-binding fragment binds to human TFR1 with a K_D of about 1×10^{-8} M or less and binds to a non-human primate (for example, cynomolgus monkey) TFR1 with a K_D of about 5×10^{-8} M or less.

[0184] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment binds to human TFR1 with a K_D ranging from about 5×10^{-9} M to about 1×10^{-12} M, from about 5×10^{-9} M to about 1×10^{-11} M, from about 5×10^{-9} M to about 1×10^{-10} M, from about 1×10^{-9} M to about 1×10^{-12} M, from about 1×10^{-9} M to about 1×10^{-11} M, or from about 1×10^{-9} M to about 1×10^{-10} M. In some embodiments, the anti-TFR1 antibody or antigen-binding fragment binds to human TFR1 with a K_D of about 9×10^{-10} M, about 8×10^{-10} M, about 7×10^{-10} M, about 6×10^{-10} M, 5×10^{-10} M, about 4×10^{-10} M, about 3×10^{-10} M, about 2×10^{-10} M, or about 1×10^{-10} M. In some embodiments, the K_D value is measured by Bio-Layer Interferometry.

TFR1-binding agents

[0185] In another aspect, provided is a TFR1-binding agent comprising the anti-TFR1 antibody or antigen-binding fragment of the present disclosure conjugated to an additional molecule. In some embodiments, the TFR1-binding agent is able to pass through the blood-brain barrier.

[0186] As used herein, “conjugating” or “conjugation” refers to two or more moieties are associated through covalent or non-covalent interactions, preferably through covalent interactions.

[0187] The additional molecule can be a protein (such as an antibody), a small compound or a nucleic acid.

[0188] In some embodiments, the anti-TFR1 antibody or antigen-binding fragment of the present disclosure can be conjugated to a therapeutic agent for treating cancer or a neurological disease or disorder.

[0189] In some embodiments, the additional molecule is an anticancer drug selected from: maytansinoid (e.g., maytansine), auristatins (e.g., MMAF, MMAE, MMAD), duostatin, cryptophycin, vinca alkaloids (e.g., vinblastine, vincristine), colchicines, aplysiatoxins, taxane, taxol, docetaxel, cabazitaxel, enediyne antibiotics, cytochalasins, camptothecins, anthracycline antibiotics (e.g., daunorubicin, dihydroxyanthracindione, doxorubicin), cytotoxic antibiotics (e.g., mitomycin, actinomycin, duocarmycin (e.g., CC-1065), auromycin, duomycin, calicheamicin, endomycin, phenomycin), adriamycin, rubidomycin, calicheamicin, cisplatin, ethidium bromide, bleomycin, mitomycin, mithramycin, pladienolide, podophyllotoxin, etoposide, mitoxantrone, 5-fluorouracil, cytarabine, gemcitabine, mercaptopurine, pentostatin, fludarabine, cladribine, nelarabine, carmustine, lomustine, methotrexate, melphalan, tenoposide and glucocorticoid.

[0190] In some embodiments, the additional molecule is a therapeutic agent for treating a disease or disorder selected from: cancer (e.g., central nervous system cancer, including brain tumor), Alzheimer's disease, Parkinson's disease, Huntington's disease, hunter syndrome (also known as "Mucopolysaccharidosis Type II", including symptoms in the CNS), schizophrenia, antidepressant, multiple sclerosis, amyotrophic lateral sclerosis, lysosomal storage disease accompanied by encephalopathy, glycogenosis, muscular dystrophy, cerebral ischemia, a prion disease, traumatic central nervous system disorder (including traumatic brain injury and traumatic spinal cord injury), viral and bacterial central nervous system disease. In some embodiments, the therapeutic agent is iduronate 2-sulfatase enzyme. In some embodiments, the therapeutic agent is an antibody for treating Alzheimer's disease.

[0191] In some embodiments, the anti-TFR1 antibody or the antigen-binding fragment of the present disclosure can be conjugated to an imaging agent, for example, for use in diagnosing cancer or a neurological disease or disorder.

[0192] An "imaging agent" is a compound that has one or more properties that allow its presence and/or location to be detected directly or indirectly. The imaging agent may be any agent used for detection, including but not limited to, a radionuclide (e.g., ^{212}Bi , ^{213}Bi , ^{11}C , ^{18}F , ^{131}I , ^{125}I , ^{111}In , ^{177}Lu , ^{13}N , ^{15}O , ^{186}Re , ^{188}Re , ^{153}Sm and ^{90}Y), biotin, a fluorescent protein, a fluorophore (e.g., FITC, Alexa Fluor 488, Alexa Fluor 568, Alexa Fluor 555, Alexa Fluor 594, Alexa Fluor 647, Cy3, Texas Red, Cy5 and Rhodamine) and an enzyme.

[0193] In some embodiments, the additional molecule is an imaging agent selected from a radionuclide, biotin, a fluorescent protein, a fluorophore, horseradish peroxidase and alkaline phosphatase.

[0194] The additional molecule (e.g., a therapeutic agent or an imaging agent) may be directly or indirectly (e.g., through a linker) linked to the anti-TFR1 antibody or antigen-binding fragment or the multispecific antibody.

[0195] The linker may comprise an active group for covalent conjugation, for example, an amine, a hydroxylamine, a maleimide group, a carboxyl group, a phenyl group, a thiol group, or a hydroxyl group.

[0196] In some embodiments, the linker is a peptide linker. A peptide linker may comprise an amino acid sequence of any length. In particular, a peptide linker may comprise an amino acid sequence of 1-50, preferably 1-30, for example 1-10 amino acids. Exemplary peptide linkers may include but not limited to polyglycines, polyalanines, polyserines and combinations thereof. Other suitable peptide linkers may include a hinge region or a functional equivalent thereof.

[0197] Other suitable linkers may be an organic compound or a polymer that is suitable for use in therapeutic proteins, for example, polyethylene glycol.

Multispecific antibodies

[0198] In yet another aspect, provided is a multispecific antibody, comprising a first antigen-binding moiety binding to TFR1, wherein the first antigen-binding moiety comprises the heavy chain variable domain binding to TFR1 as described herein. In some embodiments, the first antigen-binding moiety comprises the anti-TFR1 antibody or antigen-binding fragment of the present disclosure.

[0199] In some embodiments, the multispecific antibody further comprises a second antigen-binding moiety that binds to a second antigen. The second antigen may be an antigen different from TFR1. The second antigen may also be TFR1, and the second antigen-binding moiety binds to an epitope different from which the anti-TFR1 antibody or antigen-binding fragment of the present disclosure binds to. Various methods known in the art may be used to determine whether two antibodies bind to the same epitope, e.g., measuring whether the two antibodies compete for binding to the same epitope by ELISA, FACS or SPR.

[0200] In some preferable embodiments, the second antigen is a brain antigen. In some embodiments, the brain antigen is selected from: BACE1, amyloid precursor protein (APP), amyloid beta (A β), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein, leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), p75 neurotrophin receptor (p75NTR), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), and caspase 6.

[0201] As used herein, the term “antigen-binding moiety” refers to an amino acid sequence comprising an antigen-binding site and is capable of binding to an epitope. The definition of antigen-binding moiety falls within the definition of an antibody or antigen-binding fragment. An antigen-binding moiety may comprise one or more (e.g., 1, 2, 3, or 4) antigen-binding domains/site. In some embodiments, the antigen-binding moiety comprises one antigen-binding domains/sites. In some other embodiments, the antigen-binding moiety comprises two antigen-binding domains/sites.

[0202] The first antigen-binding moiety may be an antibody or antigen-binding fragment in any format, including but not limited to a single variable domain and a heavy-chain antibody. In some embodiments, the first antigen-binding moiety comprises the heavy chain variable domain that binds to TFR1 as described herein. In some embodiments, the multispecific antibody comprises only one heavy chain variable domain that binds to TFR1 as described herein.

[0203] The second antigen-binding moiety may be any antibody or antigen-binding fragment in any format. In some embodiments, the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region which bind to the second antigen. In some embodiments, the second antigen-binding moiety comprises a dsFv, an scFv, an scdsFv, a di-scFv, an Fab, an Fab', an scFab or an F(ab')₂. In some embodiments, the antigen-binding moiety comprises an Fab. In some embodiments, the antigen-binding moiety comprises an

F(ab')₂. In some other embodiments, the second antigen-binding moiety comprises a single variable domain binding to the second antigen, such as a VHH.

[0204] In some embodiments, the first antigen-binding moiety and the second antigen-binding moiety may be optionally linked through a linker (e.g., a peptide linker).

[0205] In some embodiments, the multispecific antibody comprises an (scFv)₂-VHH, wherein the second antigen-binding moiety comprises an (scFv)₂, and the first antigen-binding moiety comprises a VHH.

[0206] In some embodiments, the multispecific antibody further comprises an Fc region.

[0207] In some embodiments, the Fc region comprises one or more modifications to reduce the effector function. In some other embodiments, the Fc region comprises one or more modifications to enhance the effector function. In some embodiments, the effector function comprises at least one selected from ADCC, CDC and ADCP.

[0208] In some embodiments, the Fc region comprises one or more modifications to enhance the FcRn binding.

[0209] In some embodiments, the Fc region comprises one or more modifications to reduce the effector function and enhance the FcRn binding. In some embodiments, the Fc region comprises one or more modifications to enhance the effector function and enhance the FcRn binding.

[0210] In some embodiments, the Fc region is a monomeric Fc region.

[0211] In some embodiments, the Fc region is a heterodimeric Fc region comprising a first Fc region subunit and a second Fc region subunit, wherein one of the first Fc region subunit and the second Fc region subunit comprises a knob mutation, and the other comprises a hole mutation. In some embodiments, the first Fc region subunit comprises a knob mutation, and the second Fc region subunit comprises a hole mutation. In some other embodiments, the first Fc region subunit comprises a hole mutation, and the second Fc region subunit comprises a knob mutation.

[0212] In some embodiments, the knob mutation comprises one or more mutations selected from S354C and T366W. In some embodiments, the hole mutation comprises one or more mutations selected from Y349C, T366S, L368A and Y407V. In some embodiments, the knob mutation comprises S354C, and the hole mutation comprises Y349C. In some embodiments, the knob mutation comprises T366W, and the hole mutation comprises T366S, L368A and Y407V. In some particular embodiments, the knob mutation comprises S354C and T366W, and the hole mutation comprises Y349C, T366S, L368A and Y407V.

[0213] In some embodiments, the multispecific antibody comprises: a first polypeptide comprising a heavy chain variable region, a CH1 domain and a first Fc region subunit, a second polypeptide comprising a light chain variable region and a light chain constant region, and a third polypeptide comprising the heavy chain variable domain binding to TFR1 as described herein and a second Fc region subunit, wherein the heavy chain variable region and the light chain variable region bind to the second antigen, and the first Fc region subunit and the second

Fc region subunit form the heterodimeric Fc region. In some preferable embodiments, the multispecific antibody may have a configuration as shown in Figure. 12.

[0214] In some other embodiments, the multispecific antibody comprises: a first polypeptide comprising a heavy chain variable region, a CH1 domain, a first Fc region subunit, an optional peptide linker and the heavy chain variable domain binding to TFR1 as described herein, a second polypeptide comprising a heavy chain variable region, a CH1 domain and a second Fc region subunit, and a third polypeptide and a fourth polypeptide each comprising a light chain variable region and a light chain constant region, wherein the heavy chain variable region and the light chain variable region bind to the second antigen, and the first Fc region subunit and the second Fc region subunit form the heterodimeric Fc region. In some preferable embodiments, the multispecific antibody may have a configuration as shown in Figure. 13.

[0215] In some embodiments, the brain antigen is BACE1. In a particular aspect, provided is a multispecific antibody binding to TFR1 and BACE1, wherein the multispecific antibody comprises the heavy chain variable domain of the anti-TFR1 antibody of the present disclosure and an antigen-binding moiety binding to BACE1.

[0216] In some embodiments, the antigen-binding moiety binding to BACE1 comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HCDR1, an HCDR2 and an HCDR3 as comprised in the amino acid sequence of SEQ ID NO: 102, and the light chain variable region comprises an LCDR1, an LCDR2 and an LCDR3 as comprised in the amino acid sequence of SEQ ID NO: 104. In some preferable embodiments, the HCDR1 comprises an amino acid sequence of SEQ ID NO: 67, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 73, and the HCDR3 comprises an amino acid sequence of SEQ ID NO: 83; and the LCDR1 comprises an amino acid sequence of SEQ ID NO: 88, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 91, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 95.

[0217] In some particular embodiments, the antigen-binding moiety binding to BACE1 comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 102. In some particular embodiments, the antigen-binding moiety binding to BACE1 comprises a light chain variable region having the amino acid sequence of SEQ ID NO: 104. In some particular embodiments, the heavy chain variable region comprises an amino acid sequence of SEQ ID NO: 102; and the light chain variable region comprises an amino acid sequence of SEQ ID NO: 104.

[0218] In some embodiments, the multispecific antibody binding to TFR1 and BACE1 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 122.

[0219] In some embodiments, the multispecific antibody binding to TFR1 and BACE1 comprises a second polypeptide having the amino acid sequence of SEQ ID NO: 113.

[0220] In some embodiments, the multispecific antibody binding to TFR1 and BACE1 comprises a third polypeptide having the amino acid sequence of SEQ ID NO: 129, 130, 131, 132 or 133.

[0221] In some embodiments, the multispecific antibody binding to TFR1 and BACE1 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 122, a second polypeptide having the amino acid sequence of SEQ ID NO: 113, and a third polypeptide having the amino acid sequence of SEQ ID NO: 129, 130, 131, 132 or 133.

[0222] In a particular aspect, the multispecific antibody binding to TFR1 and BACE1 is Antibody PR013356 as described herein. In some embodiments, Antibody PR013356 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 52, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 24. In some embodiments, Antibody PR013356 comprises a first-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 52. In some embodiments, Antibody PR013356 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 122, a second polypeptide having the amino acid sequence of SEQ ID NO: 113, and a third polypeptide having the amino acid sequence of SEQ ID NO: 129.

[0223] In a particular aspect, the multispecific antibody binding to TFR1 and BACE1 is Antibody PR013357 as described herein. In some embodiments, Antibody PR013357 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 98, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 79. In some embodiments, Antibody PR013357 comprises a first antigen-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 98. In some embodiments, Antibody PR013357 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 122, a second polypeptide having the amino acid sequence of SEQ ID NO: 113, and a third polypeptide having the amino acid sequence of SEQ ID NO: 130.

[0224] In a particular aspect, the multispecific antibody binding to TFR1 and BACE1 is Antibody PR013358 as described herein. In some embodiments, Antibody PR013358 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 99, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 80. In some embodiments, Antibody PR013358 comprises a first antigen-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 99. In some embodiments, Antibody PR013358 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 122, a second

polypeptide having the amino acid sequence of SEQ ID NO: 113, and a third polypeptide having the amino acid sequence of SEQ ID NO: 131.

[0225] In a particular aspect, the multispecific antibody binding to TFR1 and BACE1 is Antibody PR013359 as described herein. In some embodiments, Antibody PR013359 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 100, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 81. In some embodiments, Antibody PR013359 comprises a first antigen-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 100. In some embodiments, Antibody PR013359 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 122, a second polypeptide having the amino acid sequence of SEQ ID NO: 113, and a third polypeptide having the amino acid sequence of SEQ ID NO: 132.

[0226] In a particular aspect, the multispecific antibody binding to TFR1 and BACE1 is Antibody PR013360 as described herein. In some embodiments, Antibody PR013360 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 101, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 82. In some embodiments, Antibody PR013360 comprises a first antigen-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 101. In some embodiments, Antibody PR013360 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 122, a second polypeptide having the amino acid sequence of SEQ ID NO: 113, and a third polypeptide having the amino acid sequence of SEQ ID NO: 133.

[0227] In some embodiments, the brain antigen is A β . In a particular aspect, provided is a multispecific antibody binding to TFR1 and A β , wherein the multispecific antibody comprises the heavy chain variable domain of the anti-TFR1 antibody of the present disclosure and an antigen-binding moiety binding to A β .

[0228] In some embodiments, the antigen-binding moiety binding to A β comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HCDR1, an HCDR2 and an HCDR3 as comprised in the amino acid sequence of SEQ ID NO: 103, and the light chain variable region comprises an LCDR1, an LCDR2 and an LCDR3 as comprised in the amino acid sequence of SEQ ID NO: 105. In some preferable embodiments, the HCDR1 comprises an amino acid sequence of SEQ ID NO: 68, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 74, and the HCDR3 comprises an amino acid sequence of SEQ ID NO: 84; and the LCDR1 comprises an amino acid sequence of SEQ

ID NO: 89, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 92, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 96.

[0229] In some particular embodiments, the antigen-binding moiety binding to A β comprises a heavy chain variable region having the amino acid sequence of SEQ ID NO: 103. In some particular embodiments, the antigen-binding moiety binding to A β comprises a light chain variable region having the amino acid sequence of SEQ ID NO: 105. In some particular embodiments, the heavy chain variable region comprises an amino acid sequence of SEQ ID NO: 103; and the light chain variable region comprises an amino acid sequence of SEQ ID NO: 105.

[0230] In some embodiments, the multispecific antibody binding to TFR1 and A β comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 119, 125, 126, 127 or 128.

[0231] In some embodiments, the multispecific antibody binding to TFR1 and A β comprises a second polypeptide having the amino acid sequence of SEQ ID NO: 117.

[0232] In some embodiments, the multispecific antibody binding to TFR1 and A β comprises a third polypeptide having the amino acid sequence of SEQ ID NO: 115. In some embodiments, the multispecific antibody binding to TFR1 and A β comprises a third polypeptide and a fourth polypeptide, wherein each of the third and the fourth polypeptides has the amino acid sequence of SEQ ID NO: 115.

[0233] In some embodiments, the multispecific antibody binding to TFR1 and A β comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 119, 125, 126, 127 or 128, a second polypeptide having the amino acid sequence of SEQ ID NO: 117, and a third polypeptide and a fourth polypeptide each having the amino acid sequence of SEQ ID NO: 115.

[0234] In a particular aspect, the multispecific antibody binding to TFR1 and A β is Antibody PR012932 as described herein. In some embodiments, Antibody PR012932 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 52, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 24. In some embodiments, Antibody PR012932 comprises a first-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 52. In some embodiments, Antibody PR012932 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 119, a second polypeptide having the amino acid sequence of SEQ ID NO: 117, and a third polypeptide and a fourth polypeptide each having the amino acid sequence of SEQ ID NO: 115.

[0235] In a particular aspect, the multispecific antibody binding to TFR1 and A β is Antibody PR013352 as described herein. In some embodiments, Antibody PR013352 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 98, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2

comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 79. In some embodiments, Antibody PR013352 comprises a first antigen-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 98. In some embodiments, Antibody PR013352 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 125, a second polypeptide having the amino acid sequence of SEQ ID NO: 117, and a third polypeptide and a fourth polypeptide each having the amino acid sequence of SEQ ID NO: 115.

[0236] In a particular aspect, the multispecific antibody binding to TFR1 and A β is Antibody PR013353 as described herein. In some embodiments, Antibody PR013353 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 99, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 80. In some embodiments, Antibody PR013353 comprises a first antigen-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 99. In some embodiments, Antibody PR013353 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 126, a second polypeptide having the amino acid sequence of SEQ ID NO: 117, and a third polypeptide and a fourth polypeptide each having the amino acid sequence of SEQ ID NO: 115.

[0237] In a particular aspect, the multispecific antibody binding to TFR1 and A β is Antibody PR013354 as described herein. In some embodiments, Antibody PR013354 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 100, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 81. In some embodiments, Antibody PR013354 comprises a first antigen-binding moiety comprising a heavy chain variable domain having an amino acid sequence of SEQ ID NO: 100. In some embodiments, Antibody PR013354 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 127, a second polypeptide having the amino acid sequence of SEQ ID NO: 117, and a third polypeptide and a fourth polypeptide each having the amino acid sequence of SEQ ID NO: 115.

[0238] In a particular aspect, the multispecific antibody binding to TFR1 and A β is Antibody PR013355 as described herein. In some embodiments, Antibody PR013355 comprises a first antigen-binding moiety comprising a heavy chain variable domain having a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 101, for example, a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 8, a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 16, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO: 82. In some embodiments, Antibody PR013355 comprises a first antigen-binding moiety comprising a heavy chain variable domain having an amino acid

sequence of SEQ ID NO: 101. In some embodiments, Antibody PR013355 comprises a first polypeptide having the amino acid sequence of SEQ ID NO: 128, a second polypeptide having the amino acid sequence of SEQ ID NO: 117, and a third polypeptide and a fourth polypeptide each having the amino acid sequence of SEQ ID NO: 115.

Pharmaceutical compositions

[0239] Also provided is a pharmaceutical composition comprising the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent or the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure.

[0240] The pharmaceutical composition provided herein can be in various dosage forms, e.g., in solid, semi-solid, liquid, powder, aqueous, or lyophilized forms. Depending on different dosage forms, the pharmaceutical composition could further comprise pharmaceutically acceptable excipients (see, generally, Alfonso R. Gennaro (2000) Remington: *The Science and Practice of Pharmacy*, 20th Edition. Baltimore, MD: Lippincott Williams & Wilkins).

[0241] Excipients can be used in solid dosage forms in the preparation of pharmaceutical compositions include, but are not limited to: diluents (e.g., lactose, microcrystalline cellulose and dextrose), binders and adhesives (e.g., acacia, gelatin, starch paste and carboxymethyl cellulose), lubricants (e.g., polyethylene glycol, calcium stearate and steric acid), disintegrants (e.g., starches, cellulose and cross linker polymers), preservatives, vehicles, glidants (e.g., corn starch), sweeteners (e.g., mannitol and saccharin), coating materials (e.g., povidone, ethyl cellulose and synthetic polymers), and plasticizers (e.g., castor oil, diacetylated monoglycerides, and polyethylene glycol). Excipients can be used in semi-solid dosage forms in the preparation of pharmaceutical compositions include, but are not limited to: structure forming excipients (e.g., cetostearyl alcohol and mineral oils), preservatives (e.g., benzyl alcohol, propylparaben and sodium benzoate), antioxidants (e.g., butyl hydroxyl toluene and butyl hydroxyl anisole), solubilizers (e.g., lanolin and cholesterol), gelling agents (e.g., carboxyl methyl cellulose, hydroxyl propyl cellulose and xanthan gum), and emollients (e.g., glycerin, mineral oil, petrolatum and isopropyl palmitate). Excipients can be used in liquid dosage forms in the preparation of pharmaceutical compositions include, but are not limited to: solvents (e.g., water, alcohol, acetic acid and syrups), buffers (e.g., phosphate buffers and acetate buffers), antimicrobial preservatives (e.g., benzyl alcohol, butylparaben, phenol and thiomersal), antioxidants (e.g., ascorbic acid, sodium bisulfate, thiourea and butyl hydroxy toluene), chelating agents (e.g., disodium EDTA, dihydroxy ethyl glycine and citric acid), and emulsifying agents (e.g., sodium lauryl sulfate, cetrimide and macrogol esters).

[0242] The pharmaceutical composition provided herein can be administered into a subject by any method known in the art, for example, by systemic or local administration. Routes of administration include, but are not limited to, parenteral (e.g., intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, or intracavity), topical, epidural, or mucosal (e.g. intranasal or oral). The exact dosage to be administered will depend on various factors, such as

the therapeutic objectives, the route of administration, and the condition of the subject, for example, the patient's health, body weight, sex, diet, etc. Accordingly, it will be necessary for the therapist to titer the dosage of the pharmaceutical composition and modify the route of administration as required to obtain the optimal therapeutic effect. Generally, the dosage ranges for the administration of the pharmaceutical composition provided herein are those large enough to produce desired effects.

[0243] In a preferred embodiment, the pharmaceutical composition of the present disclosure could be prepared from the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent or the multispecific antibody of the present disclosure and administered into a human subject in a therapeutically effective amount. A therapeutic dose of the antibodies could be, for example, between preferably 0.1-25 mg/kg body weight per single therapeutic dose and most preferably between 0.1-10 mg/kg body weight for single therapeutic dose. In a particular embodiment, the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent, or the multispecific antibody of the present disclosure can be formulated in accordance with conventional practice for administration by any suitable route and could generally be in a liquid form (e.g., a solution of the antibody in a sterile physiologically acceptable buffer) for administration by, for example, an intravenous, intraperitoneal, subcutaneous, or intramuscular route.

Uses and methods

[0244] In one aspect, provided is use of the anti-TFR1 antibody or antigen-binding fragment of the present disclosure in the manufacture of an agent for transporting a compound across the blood-brain barrier (BBB). In some embodiments, the agent (for example, the TFR1-binding agent, or the multispecific antibody of the present disclosure) can be used for a therapeutic or a diagnostic purpose.

[0245] Also provided is a method of transporting a compound across the blood-brain barrier in a subject, comprising conjugating the anti-TFR1 antibody or antigen-binding fragment of the present disclosure to the compound and transporting the conjugate across the blood-brain barrier.

[0246] Further provided is the anti-TFR1 antibody or antigen-binding fragment of the present disclosure for use as a medicament, wherein the anti-TFR1 antibody or antigen-binding fragment is transporting a compound across the blood-brain barrier.

[0247] The compound to be transported across the BBB may be a therapeutic agent or an imaging agent as described herein. The compound to be transported across the BBB may be conjugated to the anti-TFR1 antibody or antigen-binding fragment of the present disclosure using well known methods in the art, for example, chemical conjugation, enzymatic conjugation and recombinant DNA technology.

[0248] The anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent, the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody),

the nucleic acid or vector, or the pharmaceutical composition of the present disclosure may be used for therapy or diagnosis, for example, in the treatment of cancer or in the treatment or diagnosis of a neurological disease or disorder in a subject in need thereof.

[0249] In a particular aspect, provided is use of the anti-TFR1 antibody or antigen-binding fragment in the manufacture of a medicament for treating cancer.

[0250] Also provided is a method of treating cancer in a subject in need thereof, comprising administering a therapeutically effective amount of the anti-TFR1 antibody or antigen-binding fragment of the present disclosure to the subject.

[0251] Further provided is the anti-TFR1 antibody or antigen-binding fragment of the present disclosure for use in treating cancer.

[0252] It is reported that TFR1 expression is elevated on many different types of cancer cells, including but not limited to, solid cancers such as esophageal squamous cell carcinoma, breast cancer, ovarian cancer, lung cancer, cervical cancer, bladder cancer, osteosarcoma, pancreatic cancers, bile duct cancer, renal cell carcinoma, hepatocellular carcinoma, adrenal cortical carcinoma, and cancers of the nervous system as well as hematopoietic malignancies such as acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and non-Hodgkin lymphoma (NHL). Furthermore, the extracellular accessibility, ability to internalize, and central role in cancer cell pathology of TFR1 make it an attractive target for antibody-mediated therapy.

[0253] The anti-TFR1 antibody or antigen-binding fragment of the present disclosure may be used for cancer therapy, for example, by (1) indirectly through conjugating to an anticancer drug (for example, the TFR1-binding agent comprising an anticancer drug as described herein) and/or (2) directly through fusing to an Fc region to induce Fc effector functions, such as ADCC, ADCP, and/or CDC. In some embodiments, the cancer is a central nervous system cancer.

[0254] In some embodiments, the cancer comprises cancer cells overexpressing TFR1. In some embodiments, the cancer is selected from esophageal squamous cell carcinoma, breast cancer, ovarian cancer, lung cancer, cervical cancer, bladder cancer, osteosarcoma, pancreatic cancers, bile duct cancer, renal cell carcinoma, hepatocellular carcinoma, adrenal cortical carcinoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, leukemia (e.g., acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), chronic myelogenous leukemia, chronic lymphoblastic leukemia, and chronic lymphoid leukemia) and multiple myeloma.

[0255] In a particular aspect, provided is use of the anti-TFR1 antibody or antigen-binding fragment in the manufacture of a medicament for treating a neurological disease or disorder, wherein the anti-TFR1 antibody or antigen-binding fragment is conjugated to a therapeutic agent for treating the neurological disease or disorder. In some embodiments, the therapeutic agent comprises the second antigen-binding moiety binding to BACE1 or A β as described herein.

[0256] Also provided is a method of treating a neurological disease or disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the anti-TFR1

antibody or antigen-binding fragment of the present disclosure conjugated to a therapeutic agent for treating the neurological disease or disorder to the subject.

[0257] Further provided is the anti-TFR1 antibody or antigen-binding fragment of the present disclosure for use as a medicament for treating a neurological disease or disorder, wherein the anti-TFR1 antibody or antigen-binding fragment is conjugated to a therapeutic agent for treating the neurological disease or disorder.

[0258] The multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure may be used for treating a disease or disorder associated with the second antigen and/or detecting the second antigen in an isolated biological sample or in situ. In some embodiments, the multispecific antibody may be conjugated to an imaging agent as described herein. For example, the multispecific antibody may be conjugated to a radionuclide (e.g., a positron emission tomography (PET) radiotracer, such as ^{11}C , ^{18}F , ^{13}N and ^{15}O) to form a radioimmunoconjugate for in situ detection of the second antigen, for example, for use in diagnosing a disease or disorder associated with the second antigen.

[0259] In a particular aspect, provided is use of the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure in the manufacture of a medicament for treating a neurological disease or disorder.

[0260] Also provided is a method of treating a neurological disease or disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure.

[0261] Further provided is the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure for use in treating a neurological disease or disorder.

[0262] In some embodiments, the neurological disease or disorder is selected from Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury and glaucoma. In some embodiments, the neurological disease or disorder is Alzheimer's disease.

[0263] In yet another particular aspect, provided is use of the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody of the present disclosure in the manufacture of an agent or a kit for diagnosing Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury or glaucoma. In some preferable embodiments, the agent or the kit is for use in situ.

[0264] Further provided is the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure for use in diagnosing Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury or glaucoma.

[0265] Amyloid plaques are common features of and play a central role in many neurodegenerative diseases, including Alzheimer's disease. The multispecific antibody (e.g.,

the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure may be useful in inhibiting the formation of amyloid plaques and/or removing existing amyloid plaques in the brain.

[0266] Accordingly, in yet another particular aspect, provided is use of the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure in the manufacture of a medicament for inhibiting and/or decreasing the formation of amyloid plaques in the brain.

[0267] Also provided is a method of inhibiting and/or decreasing the formation of amyloid plaques in the brain of a subject in need thereof, comprising administering a therapeutically effective amount of the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) of the present disclosure to the subject to inhibit and/or decrease the formation of amyloid plaques in the brain.

[0268] Further provided is the multispecific antibody (e.g., the anti-TFR1/BACE1 antibody or the anti-TFR1/A β antibody) for use in inhibiting and/or decreasing the formation of amyloid plaques in the brain.

Nucleic acids, vectors and host cells

[0269] In another aspect, provided is a nucleic acid encoding the anti-TFR1 antibody or antigen-binding fragment or the multispecific antibody of the present disclosure. In some embodiments, the nucleic acid of the present disclosure is an isolated nucleic acid.

[0270] In yet another aspect, provided is a vector comprising the nucleic acid of the present disclosure. In some embodiments, the vector is an expression vector, such as a plasmid for expression in bacteria, yeast or mammalian cells, or a phage vector and a phagemid vector for phage display. The expression vector may further comprise additional polynucleotide sequences, such as regulatory sequences and antibiotic resistance genes.

[0271] The nucleic acid of the present disclosure may be present in one or more vectors.

[0272] The nucleic acid of the present disclosure may be obtained by using various methods known in the art. For example, the nucleic acid of the present disclosure may be isolated from a phage display library, a yeast display library, an immunized animal (e.g., a transgenic mouse, or a camelid), an immortalized cell (e.g., a mouse B-cell hybridoma, an EBV-immortalized B cell) or chemically synthesized. The nucleic acid of the present disclosure may be codon-optimized for optimal expression in a host cell.

[0273] In some embodiments, the nucleic acid of the present disclosure is prepared as a recombinant nucleic acid comprising additional nucleic acid sequences, such as regulatory elements and nucleic acid sequences encoding desired peptides or proteins. Recombinant nucleic acids can be prepared using molecular cloning techniques well known in the art, for example, chemical synthesis, site-directed mutagenesis and polymerase chain reaction (PCR) techniques (see Sambrook, J., E. F. Fritsch, and T. Maniatis. (1989). *Molecular cloning: a laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

Regulatory elements suitable for use in the present disclosure may include, but are not limited to, enhancers, insulators, internal ribosome entry sites (IRES). Examples of peptides or proteins that may facilitate the detection or isolation of the expressed antibody or antigen-binding fragment, include, but are not limited to, affinity tags (e.g., a biotin tag, a polyhistidine-tag (His₆) or a Glutathione S-Transferase (GSH) tag), enzyme-cleavable peptides, and reporter proteins (e.g., fluorescent proteins). In some embodiments, nucleic acids of the present disclosure and the regulatory elements and/or nucleic acid sequences encoding desired peptides or proteins can be operably linked to a promoter. Depending on the type of host cells and purification strategies to be used, those of skill in the art are able to select suitable expression vectors, promoters, regulatory elements, and peptides or proteins.

[0274] In another aspect, provided is a host cell comprising the nucleic acid or the vector of the present disclosure.

[0275] A host cell herein can be used to produce the anti-TFR1 antibody or antigen-binding fragment or the multispecific antibody of the present disclosure. Examples of host cells for producing recombinant antibodies may include but are not limited to: mammalian cells, for example, HEK293 cells, Chinese Hamster Ovary (CHO) cells, COS cells, myeloma cells, baby hamster kidney (BHK), Hela and Vero cells; insect cells, for example, sf9, sf21 and Tn5; plant cells, for example plants belonging to the genus *Nicotiana* (e.g. *Nicotiana tabacum*); yeast cells, for example, those belonging to the genus *Saccharomyces* (e.g. *Saccharomyces cerevisiae*); and bacterial cells, for example *Escherichia coli* or *Bacillus subtilis*, etc.

[0276] The nucleic acid or vector may be introduced into a suitable host cell using well known methods in the art. Such methods may include but are not limited to, liposome transfection, electroporation, viral transduction and calcium phosphate transfection.

[0277] In some embodiments, a single vector comprising the nucleic acids encoding both heavy and light chains could be used. In a particular embodiment, two vectors are used, wherein one vector encodes the light chain and the other encodes the heavy chain of said antibody. In some embodiments, the expression vector(s) could further contain one or more selective marker genes, for example, a neomycin or puromycin resistance gene. In some embodiments, chaperon plasmids may be introduced into the same host cell together with the expression vectors described above, for example, in bacteria, to assist the solubilisation and/or folding of the antibodies. Isolation and purification of antibodies may be performed using well-known techniques in the art, for example, using a Protein A affinity column.

[0278] Also provided is a method of producing the anti-TFR1 antibody or antigen-binding fragment or the multispecific antibody of the present disclosure, comprising: culturing the host cell of the present disclosure under suitable conditions to allow the expression of the anti-TFR1 antibody or antigen-binding fragment or the multispecific antibody, and optionally isolating the anti-TFR1 antibody or antigen-binding fragment or the multispecific antibody from the host cell or the culture medium.

Kits

[0279] Also provided is a kit comprising the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent, the multispecific antibody, the nucleic acid or vector, or the pharmaceutical composition of the present disclosure, and optionally instructions for use thereof. The kit may be used for treatment or diagnosis purposes. In some embodiments, the kit is used in a method of detecting the second antigen as described herein in an isolated biological sample or in situ.

[0280] The kit may further comprise a suitable container. In some embodiments, the kit further comprises a device for administration. Typically, a kit may further comprise a label, which indicates the intended use and/or method of use of the contents in the container. The term “label” includes any written or documented material provided on or with the kit or otherwise provided with the kit.

[0281] The present disclosure provides the following exemplary embodiments:

[0282] Embodiment 1. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 24, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0283] Embodiment 2. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 79, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0284] Embodiment 3. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 80, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0285] Embodiment 4. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 81, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0286] Embodiment 5. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 82, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0287] Embodiment 6. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 52.

[0288] Embodiment 7. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 98.

[0289] Embodiment 8. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 99.

[0290] Embodiment 9. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 100.

[0291] Embodiment 10. An antibody or antigen-binding protein comprising an antigen-binding fragment, wherein the antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 101.

[0292] Embodiment 11. An antibody or antigen-binding protein comprising a polypeptide with the amino acid sequence set forth in SEQ ID NO: 59.

[0293] Embodiment 12. An antibody or antigen-binding protein comprising a polypeptide with the amino acid sequence set forth in SEQ ID NO: 107.

[0294] Embodiment 13. An antibody or antigen-binding protein comprising a polypeptide with the amino acid sequence set forth in SEQ ID NO: 108.

[0295] Embodiment 14. An antibody or antigen-binding protein comprising a polypeptide with the amino acid sequence set forth in SEQ ID NO: 109.

[0296] Embodiment 15. An antibody or antigen-binding protein comprising a polypeptide with the amino acid sequence set forth in SEQ ID NO: 110.

[0297] Embodiment 16. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 24, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 88, 91 and 95, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 67, 73 and 83, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0298] Embodiment 17. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a

heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 79, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 88, 91 and 95, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 67, 73 and 83, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0299] Embodiment 18. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 80, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 88, 91 and 95, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 67, 73 and 83, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0300] Embodiment 19. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 81, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 88, 91 and 95, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 67, 73 and 83, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0301] Embodiment 20. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 82, respectively; the second antigen-binding fragment thereof

comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 88, 91 and 95, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 67, 73 and 83, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0302] Embodiment 21. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 24, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 89, 92 and 96, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 68, 74 and 84, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0303] Embodiment 22. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 79, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 89, 92 and 96, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 68, 74 and 84, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0304] Embodiment 23. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 80, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 89, 92 and 96, respectively, the

VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 68, 74 and 84, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0305] Embodiment 24. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 81, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 89, 92 and 96, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 68, 74 and 84, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0306] Embodiment 25. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 8, 16 and 82, respectively; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises an LCDR1, an LCDR2 and an LCDR3, wherein the LCDR1, LCDR2 and LCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 89, 92 and 96, respectively, the VH region comprises an HCDR1, an HCDR2 and an HCDR3, wherein the HCDR1, HCDR2 and HCDR3 comprise the amino acid sequences set forth in SEQ ID NOs: 68, 74 and 84, respectively; and wherein the CDR regions are defined as Chothia numbering system.

[0307] Embodiment 26. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 52; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 104, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 102.

[0308] Embodiment 27. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 98; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the

amino acid sequence set forth in SEQ ID NO: 104, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 102.

[0309] Embodiment 28. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 99; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 104, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 102.

[0310] Embodiment 29. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 100; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 104, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 102.

[0311] Embodiment 30. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 101; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 104, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 102.

[0312] Embodiment 31. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 52; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 105, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 103.

[0313] Embodiment 32. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 98; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 105, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 103.

[0314] Embodiment 33. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a

heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 99; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 105, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 103.

[0315] Embodiment 34. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 100; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 105, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 103.

[0316] Embodiment 35. A bispecific antibody or antigen-binding protein comprising two different antigen-binding fragments, the first antigen-binding fragment thereof comprises a heavy chain variable region (VH), the VH region comprises the amino acid sequence set forth in SEQ ID NO: 101; the second antigen-binding fragment thereof comprises a light chain variable region (VL) and a heavy chain variable region (VH), the VL region comprises the amino acid sequence set forth in SEQ ID NO: 105, the VH region comprises the amino acid sequence set forth in SEQ ID NO: 103.

[0317] Embodiment 36. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 122, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 113, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 129.

[0318] Embodiment 37. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 122, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 113, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 130.

[0319] Embodiment 38. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 122, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 113, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 131.

[0320] Embodiment 39. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 122, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 113, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 132.

[0321] Embodiment 40. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 122, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 113, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 133.

[0322] Embodiment 41. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 119, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 117, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 115.

[0323] Embodiment 42. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 125, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 117, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 115.

[0324] Embodiment 43. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 126, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 117, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 115.

[0325] Embodiment 44. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 127, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 117, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 115.

[0326] Embodiment 45. A bispecific antibody or antigen-binding protein comprising three polypeptides, wherein, the first polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 128, the second polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 117, and the third polypeptide comprises the amino acid sequence set forth in SEQ ID NO: 115.

[0327] Although the present disclosure has been described with reference to particular embodiments, it is to be understood that these embodiments are merely illustrative of the application and principles of the present disclosure. Modifications can be made without departing from the spirit and scope of the present disclosure.

Beneficial Effects

[0328] The beneficial effects of the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent, or the multispecific antibody of the present disclosure may include at least one of the following:

(1) the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent, or the multispecific antibody of the present disclosure binds to human TFR1 and non-human primate TFR1;

(2) the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent, or the multispecific antibody of the present disclosure does not substantially affect binding of transferrin to TFR1;

(3) the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent, or the multispecific antibody of the present disclosure binds to the transferrin-TFR1 complex; and

(4) the anti-TFR1 antibody or antigen-binding fragment, the TFR1-binding agent, or the multispecific antibody of the present disclosure exhibit TFR1-binding characteristics (e.g., binding affinity to hTFR1 and dissociation from hTFR1) that allow for efficient BBB crossing and a high relative uptake in the brain.

[0329] Further, binding of the anti-TFR1 antibody or antigen-binding fragment of the present disclosure to TFR1 requires only a single variable domain which has a low molecular weight favorable for production, synthesis and BBB crossing.

[0330] The anti-TFR1 antibody or antigen-binding fragment of the present disclosure can be a fully human antibody. Therapeutic antibodies produced in non-human systems could be possibly immunogenic and elicit undesirable and sometimes fatal immune reactions, for example, a murine antibody often induces a human anti-mouse antibody response (HAMA). In addition, immunogenicity can also alter the pharmacokinetics of a therapeutic antibody by impacting clearance, thereby affecting the *in vivo* efficacy.

EXAMPLES

[0331] The present disclosure is further described below with reference to specific examples. It is to be understood that the examples are not intended to limit the scope of the disclosure. The specific experimental methods which were not mentioned in the following examples were carried out according to conventional experimental method. Unless otherwise specified, instruments described could be obtained commercially, and reagents and materials could be obtained commercially or prepared according to methods known in the art.

Example 1: Preparation of Immunogen

[0332] Human TFR1 mRNA-LNP was used as the immunogen. Human TFR1 mRNA was produced *in vitro* using T7 RNA polymerase-mediated transcription from a linearized DNA template, which encodes hTFR1 (uniprot no.: P02786) and incorporates the 5' and 3' untranslated regions and a poly-A tail. hTFR1 mRNA was then encapsulated in lipid nanoparticles. Lipid-nanoparticle (LNP) formulations were prepared by mixing ethanol and aqueous phases at a 1:3 volumetric ratio in a microfluidic device. The ethanol phase was prepared by solubilizing a mixture of ionizable lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and PEG-lipid at a molar ratio of 50:10:38.5:1.5. The

aqueous phase was prepared in citrate buffer with hTFR1 mRNA. LNPs were dialyzed against PBS and concentrated to desired concentrations.

Example 2: Animal Immunization Schemes

[0333] To obtain TFR1-specific antibodies (referred to as anti-TFR1 antibody), Harbour HCAb transgenic mice (<https://harbourantibodies.com/>; see for example WO2007/096779 for detailed description) were immunized with human TFR1 mRNA-LNP as the immunogen. The immunization scheme is listed in Table 1 below. In brief, each mouse was administrated with 80 µg of the immunogen for the prime vaccination and 50 µg for following boosters via subcutaneous injection (s.c.). The immunization was conducted with immunogen diluted in PBS every three weeks for a total of 5 times. Serum titers were tested against human TFR1-His protein (Acro, Catalog # CD1-H5243) using ELISA.

[0334] Table 1. Immunization scheme

Immunogen	Animal No.	Strain	Route	Dosage (µg/animal)
Human TFR1 mRNA-LNP	10	HCAb 2.1	s.c.	80/50/50/50/50

Example 3: TFR1 HCAb Antibody Discovery

[0335] In this example, spleen from mice with high antibody titers were harvested to prepare cDNA. The variable regions of HCAb cDNA were amplified by PCR using specific primers (5'-GGTGTCCAGTGTGTSAGGTRCAGCTG-3', SEQ ID NO: 134; 5'-AATCCCTGGGCACTGARGAGACGGTGACCRKKGT-3', SEQ ID NO: 135) and cloned on mammalian expression vector (pCAG) which contains human immunoglobulin heavy chain Fc region of the IgG1 subclass to generate HCAb cDNA libraries (name as "pCAG-HCAb libraries"). The plasmids of pCAG-HCAb libraries were prepared and transfected into HEK293 cells (ATCC, Catalog # CRL-1573) on 96-well plates for expression, then the supernatants of HEK293-pCAG-HCAb were harvested and transferred to different 96-well plates for screening by *in vitro* binding assay. Binding to recombinant human TFR1 His-tag protein (Acro, Catalog # CD1-H5243) were tested by ELISA. Binding to stable cell line HEK293T-huTFR1 expressing human TFR1 and stable cell line HEK293T-cynoTFR1 expressing cynomolgus monkey TFR1 (UniProtKB/TrEMBL: G8F602) were tested by FACS. HEK293 cell supernatants which exhibited binding to recombinant human TFR1 His-tag protein, HEK293T-huTFR1 and HEK293T-cynoTFR1 were selected for sequencing. Finally, multiple HCAb clones were selected for the further characterization.

Example 4: Antibody Production and Purification

[0336] The recombinant plasmids encoding target antibodies were transiently transfected into HEK293-6E cells (National Research Council) using PEI (Polyscience, Catalog # 24885). After transfection, the cells were incubated at 37°C with 5% CO₂ and shaking at 120 rpm. The cell culture supernatants containing target antibodies were harvested 6-7 days post-transfection by centrifugation and filtration. Monoclonal antibodies were purified using Protein A magnetic beads (AmMag Protein A Magnetic Beads, Genscript, Catalog # L00695).

[0337] The purity of the antibodies was tested by SEC-HPLC (Agilent 1260 Infinity II HPLC with Welch Xtimate SEC-300 Culum, 1× PBS pH 7.4 as mobile phase) and SDS-PAGE (SurePAGE, Bis-Tris, 10×8, 4-12%, 12 wells, Genscript, Catalog # M00653). HCAb antibody PR012592 were successfully expressed and purified for further characterization. The amino acid sequences of PR012592 are shown in Table 2 (Chothia defined CDRs).

[0338] Table 2. The amino acid sequences of anti-TFR1 antibody (PR012592)

SEQ ID NO:	PR012592	anti-TFR RP010P011A09 HCAb hIgG1(C220S)
59	PR012592 Heavy Chain	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWI RQAPGKGLEWVSYISTSGSTMYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAIYYCARKEDSNAWAGGFEI WGQGTMTVTSSEPKSSDKTHTCPPCPAPELLGSPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGK
52	PR012592 VH	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWI RQAPGKGLEWVSYISTSGSTMYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAIYYCARKEDSNAWAGGFEI WGQGTMTVTVSS
4	PR012592 VH FWR1 (Chothia)	EVQLMESGGGLVKPGGSLRLSCVAS
8	PR012592 VH CDR1 (Chothia)	GFTFSDY
12	PR012592 VH FWR2 (Chothia)	YMSWIRQAPGKGLEWVSYI
16	PR012592 VH CDR2 (Chothia)	STSGST

20	PR012592 VH FWR3 (Chothia)	MYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAIY YCAR
24	PR012592 VH CDR3 (Chothia)	KEDSNAWAGGFEI
27	PR012592 VH FWR4 (Chothia)	WGQGTMTVTVSS

[0339] In the meantime, anti-TFR1 antibodies PR011073, PR011074 and PR012560 as reference were produced following the procedures showed above with sequence information from US10323089B2 (Clone 299; named PR011073 in this disclosure), US20210145978A1 (Clone 21.12; named PR011074 in this disclosure) and US20220064288A1 (JR141; named PR012560 in this disclosure), respectively, as listed in Table 3.

[0340] Table 3. SEQ ID NOs for the amino acid sequences of anti-TFR1 reference antibodies used in this application

Antibody ID	light chain	heavy chain	VL	VH	Source of VL and VH
PR011073	60	56	53	49	Clone 299 from US10323089B2
PR011074	61	57	54	50	Clone 21.12 from US20210145978A1
PR012560	62	58	55	51	JR141 from US20220064288A1

Example 5: Binding Affinities of anti-TFR1 Antibodies to TFR1

5.1 Binding affinity to human and cyno TFR1-His proteins

[0341] Binding of recombinant anti-TFR1 antibodies to human TFR1-His protein (Acro, Catalog # CD1-H5243) and cyno TFR1-His protein (Acro, Catalog # TFR-C524a) were tested by ELISA. In brief, 1 µg/mL antigen was added to 96-well plates at 100 µL/well. After overnight incubation at 4°C, the plates were washed with 1× PBST (300 µL/well) for 3 times, and then blocked with 5% non-fat milk in 1× PBST (300 µL/well) at 37°C for 1 hour. Anti-TFR1 antibodies were serially diluted in PBS and then added to the plates. After 1-hour incubation at 37°C, the plates were washed with 1× PBST (300 µL/well) for 3 times, and added with secondary antibody (Goat anti-Human IgG-Fc Fragment HRP Conjugated, BETHYL, A80-304P) at 100 µL/well. After 1-hour incubation at 37°C, the plates were washed with 1× PBST (300 µL/well) for 3 times, and added with TMB at 100 µL/well for about 5 min. Finally, the plates were added with 100 µL/well 2 M H₂SO₄ to stop the reaction and read with Molecular device spectra max plus384 at 450 nm.

[0342] The results are shown in Figure 1, Figure 2 and Table 4. The results indicate that anti-TFR1 HCAb PR012592 shows strong binding affinity to human and cyno TFR1-His proteins. These results indicate that anti-TFR1 HCAb PR012592 binds to human and cyno TFR1 with high affinity.

[0343] Table 4. Binding affinities of anti-TFR1 antibodies to human and cyno TFR1-His proteins measured by ELISA

Antigen	Antibody ID	EC ₅₀ (nM)
human TFR1-His protein	PR012592	0.2184
	PR012560	0.1089
Cyno TFR1-His protein	PR012592	0.1682
	PR012560	4.811

5.2 Binding affinity to TFR1-expressing cells

[0344] Binding of anti-TFR1 antibodies to human (HEK293T-huTFR1) or cynomolgus (HEK293T-cynoTFR1) TFR1-overexpressing cells was tested by flow cytometry.

[0345] In brief, anti-TFR1 antibodies were serially diluted in PBS. Antibody solution was incubated with 1×10^5 cells at 4°C for 1 hour. The cells were washed twice with PBS, and 100 μ L of 1:1000 diluted fluorescently labelled anti-human IgG antibody (Alexa Fluor 647 AffiniPure Goat Anti-Human IgG Fc, Jackson ImmunoResearch, Catalog 109-606-008) was added into each well. After 1-hour incubation at 4°C, cells were washed twice with staining buffer and subjected to flow cytometry. The fluorescent signals are presented as median fluorescence intensity (MFI).

[0346] The results are shown in Figure 3, Figure 4 and Table 5. These results show that anti-TFR1 HCAb PR012592 has a strong binding affinity to HEK293T-huTFR1 cells and HEK293T-cynoTFR1 cells, indicating that anti-TFR1 HCAb PR012592 has cross-reactivity with cynoTFR1.

[0347] Table 5. Binding affinities of anti-TFR1 antibodies to HEK293T-huTFR1 and HEK293T-cynoTFR1 cells measured by FACS

Target Cell	Antibody ID	EC ₅₀	MFI Max
HEK293T-huTFR1 cells	PR012592	11.00	114448
	PR012560	4.811	110715
HEK293T-cynoTFR1 cells	PR012592	10.97	194203
	PR012560	6.529	129065

5.3 Binding Affinity to CHOK1-hCD40 Cells

[0348] Binding of anti-TFR1 antibodies to CHOK1-hCD40 cells was tested by flow cytometry using similar procedures as described in Example 5.2. In this example, hCD40-expressing cell lines are CHOK1 cell lines that had been transfected to express human CD40 (hCD40) on the surface.

[0349] The results are shown in Figure 5. These results show that anti-TFR1 HCAb PR012592 has no binding affinity to CHOK1-hCD40 cells, indicating that this HCAb antibody has no cross-reactivity with human CD40.

Example 6: Epitope Binning by Competition Assay

[0350] To determine whether anti-TFR1 antibodies bind to human TFR1 on different or approximate binding epitopes, flow cytometry was used to perform epitope competition experiments on the anti-TFR1 antibodies.

[0351] In brief, antibodies to be tested (anti-TFR1 antibodies or human IgG1 as negative control) were serially diluted in PBS containing 2 µg/mL biotin-PR12560. Antibody solution was incubated with 1×10^5 HEK293T-huTFR1 cells at 4°C for 1 hour. The cells were washed twice with PBS, and 100 µL of 1:1000 diluted fluorescently labelled Streptavidin (Alexa Fluor 647-conjugated Streptavidin, Jackson ImmunoResearch, Catalog # 016-600-084) was added into each well. After 30 min incubation at 4°C, cells were washed twice with staining buffer and subjected to flow cytometry. The inhibition rate is calculated by the following formula:

[0352] Inhibition rate (%) = $(A-B)/(A-C) \times 100$

A: 100% signal of no antibody treatment;

[0353] B: 100% signal of each tested antibody;

C: the signals of only Streptavidin.

[0354] As shown in Figure 6, the results show that HCAb antibody PR012592 and PR012560 share similar or overlapping epitopes.

Example 7: Inhibition Activity of anti-TFR1 Antibodies on Human TF Protein Binding to Human TFR1**7.1 ELISA**

[0355] To study the activity of anti-TFR1 antibodies in blocking the binding of human TF to its receptor human TFR1 *in vitro* by ELISA, human TFR1-His protein was used to perform protein-level human TF/human TFR1 binding and blocking experiments.

[0356] In brief, 0.5 µg/mL human TF-His protein (Acro, Catalog # TRN-H82E3) was added to the 96-well plate at 100 µL each well. After overnight incubation at 4°C, the plates were washed with 1× PBST (300 µL/well) for 3 times, and then blocked with 5% non-fat milk in 1× PBST (300 µL/well) at 37°C for 2 hours. Subsequently, the plates were washed for 3 times, and added with 0.5 µg/mL human TFR1-His protein and incubated at 37°C for 1 hour. Then the plates were washed for 3 times, and added with anti-TFR1 antibodies serially diluted in PBS. After 1-hour incubation at 37°C, the plates were washed for 3 times, added with secondary antibody (Goat anti-Human IgG-Fc Fragment HRP Conjugated, BETHYL, A80-304P) at 100 µL/well. After 1-hour incubation at 37°C, the plates were washed for 6 times, and then added with 100 µL/well TMB for about 5 min. Finally, the plates were added with 100 µL/well 2 M H₂SO₄ to stop the reaction and read with Molecular device spectra max plus384 at 450 nm.

[0357] The results are shown in Figure 7. The results show that PR011074 could not bind to the TF-TFR complex, indicating that the epitope for PR011074 is located within the TF binding region. The binding signal of PR011073 to the TF-TFR complex was very low, indicating that the epitope for PR011073 overlaps with the TF binding region. PR12560 and PR012592

exhibited strong binding ability to the TF-TFR complex, indicating that the epitopes for these antibodies are distant from the TF binding region.

7.2 FACS

[0358] To study the activity of anti-TFR1 antibodies in blocking the binding of human TF to its receptor human TFR1 *in vitro* by flow cytometry, HEK293T-huTFR1 cells were used to perform cell-based human TF/human TFR1 binding and blocking experiments.

[0359] In brief, antibodies to be tested (anti-TFR1 antibodies or human IgG1 as negative control; final concentration 4 nM, 20 nM or 100 nM) and Biotinylated human TF-His protein (Acro, Catalog # TRN-H82E3) (final concentration 0.1 µg/mL) were incubated with 1×10^5 cells at 4°C for 1 hour. The cells were washed twice with PBS, and 100 µL of 1:200 diluted fluorescently labelled Streptavidin (eBioscience™ Streptavidin PE Conjugate, Invitrogen™, Catalog # 12-4317-87) was added into each well. After 30 min incubation at 4°C, cells were washed twice with staining buffer and subjected to flow cytometry. The inhibition rate is calculated by the following formula:

[0360] Inhibition rate (%) = $(A-B)/(A-C) \times 100$

A: 100% signal of no antibody treatment;

B: 100% signal of each tested antibody;

C: the signals of only Streptavidin.

[0361] The results are shown in Figure 8. PR011074 strongly blocked the binding of human TF to its receptor. PR011073 and PR012560 enhanced the binding of human TF to its receptor. At different concentrations, the inhibition rate tested for PR012592 was less than 10%. These results show that compared with the control antibodies (PR011073, PR011074 and PR012560), PR012592 neither inhibits nor increases the binding of TF to its receptor at different concentrations, indicating that PR012592 does not affect the function of TF binding to its receptor at all.

Example 8: Binding Affinity to TFR1 Proteins by BLI Method

[0362] In BLI (Bio-Layer Interferometry) analysis, anti-TFR1 antibodies were diluted to 5 µg/mL using freshly prepared 1× kinetic buffer (10× kinetic buffer (ForteBio, #18-1105) was diluted with PBS (BBI Life Sciences, #E607016-0500)) and captured on the surface of anti-human Fc (AHC) Octet biosensors (ForteBio, #18-5060) to reach capture levels between 0.8-1.0 nM. The captured biosensors were then dipped in wells containing 2-fold serial dilutions of antigen proteins to detect association signals, followed by dissociation steps in wells containing 1× kinetic buffer. Human and cyno TFR1-His proteins were diluted from 80 nM to 20 nM; the association phase was 180 seconds, and the dissociation phase was 600 seconds. The sensorgrams were recorded and the reference signals were subtracted before curve fitting using ForteBio Data Analysis 11.0 software. Association rates (k_{on}) and dissociation rates (k_{dis}) were calculated using a simple one-to-one Langmuir binding model. The equilibrium

dissociation constant (K_D) was calculated as the ratio of k_{dis}/k_{on} . The binding kinetics parameters for anti-TFR1 antibodies binding to human TFR1 and cyno TFR1 are summarized in Table 6.

[0363] Table 6. Kinetics of anti-TFR1 antibodies to soluble human and cyno TFR1 proteins

Antibody ID	TFR1 protein conc. (nM)	Human TFR1 K_D (M)	Cyno TFR1 K_D (M)
PR012592	20-80	<1.0E-12	<1.0E-12
PR012560		4.51E-12	1.10E-09

Example 9: Antibody Engineering for PR012592

[0364] PR012592 was modified by alanine scanning in the CDR region to reduce its binding affinity to human TFR1. PR012592-derived mutants were produced by transient expression using the procedures as described in Example 4. The binding activities of the mutants to TFR1 were tested by ELISA and Fortebio Octet, and several mutants were selected for further evaluation according to their diverse binding affinity to TFR1. The amino acid sequences of PR012592-derived mutants are shown in Table 7 and Table 8 (Chothia defined CDRs).

[0365] Table 7. SEQ ID NOs for the amino acid sequences of PR012592 and its mutants

Antibody ID	Mutation	Heavy Chain	VH	HCDR1	HCDR2	HCDR3
PR012592	Parental	59	52	8	16	24
PR012838	D101A	107	98	8	16	79
PR012839	W105A	108	99	8	16	80
PR013071	E100A	109	100	8	16	81
PR013072	N103A	110	101	8	16	82

[0366] Table 8. The amino acid sequences of PR012592-derived mutants

SEQ ID NO:	PR012838	anti-TFR PR012592_D101A HCAb hIgG1(C220S,AAA)
107	PR012838 Heavy Chain	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWIR QAPGKGLEWVSYISTSGSTMYADSVKGRFTISRDNK NSLYLQMNSLRAEDTAIYYCARKEASNAWAGGFEIWG QGTMVTVSSEPKSSDKTHTCPPCPAPEAAGAPSVFLFP PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE

		NNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
98	PR012838 VH	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWIR QAPGKGLEWVSYISTSGSTMYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAIYYCARKEASNAWAGGFEIW GQGTMTVTSS
4	PR012838 VH FWR1 (Chothia)	EVQLMESGGGLVKPGGSLRLSCVAS
8	PR012838 VH CDR1 (Chothia)	GFTFSDY
12	PR012838 VH FWR2 (Chothia)	YMSWIRQAPGKGLEWVSYI
16	PR012838 VH CDR2 (Chothia)	STSGST
20	PR012838 VH FWR3 (Chothia)	MYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAIYY CAR
79	PR012838 VH CDR3 (Chothia)	KEASNAWAGGFEI
27	PR012838 VH FWR4 (Chothia)	WGQGTMTVTSS
SEQ ID NO:	PR012839	anti-TFR PR012592_W105A HCAb hIgG1(C220S,AAA)
108	PR012839 Heavy Chain	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWIR QAPGKGLEWVSYISTSGSTMYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAIYYCARKEDSNAAGGFEIW GQGTMTVTSSEPKSSDKTHTCPPCPAPEAAGAPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FKNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT VTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSD IAVEWESNGQPENNYKTTTPVLDSGSEFFLYSKL TVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
99	PR012839 VH	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWIR QAPGKGLEWVSYISTSGSTMYADSVKGRFTISRDN AKNSLYLQMNSLRAEDTAIYYCARKEDSNAAGGFEIW GQGTMTVTSS

4	PR012839 VH FWR1 (Chothia)	EVQLMESGGGLVKPGGSLRLSCVAS
8	PR012839 VH CDR1 (Chothia)	GFTFSDY
12	PR012839 VH FWR2 (Chothia)	YMSWIRQAPGKGLEWVSYI
16	PR012839 VH CDR2 (Chothia)	STSGST
20	PR012839 VH FWR3 (Chothia)	MYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAIYY CAR
80	PR012839 VH CDR3 (Chothia)	KEDSNAAAGGFEI
27	PR012839 VH FWR4 (Chothia)	WGQGTMTVTSS
SEQ ID NO:	PR013071	anti-TFR PR012592_E100A HCAb hIgG1(C220S,AAA)
109	PR013071 Heavy Chain	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWIR QAPGKGLEWVSYISTSGSTMYYADSVKGRFTISRDNANK NSLYLQMNSLRAEDTAIYYCARKADSNAAWAGGFEIWG QGTMTVTSSEPKSSDKTHTCPPCPAPEAAGAPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPV LDSGDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHN HYTQKSLSLSPGK
100	PR013071 VH	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWIR QAPGKGLEWVSYISTSGSTMYYADSVKGRFTISRDNANK NSLYLQMNSLRAEDTAIYYCARKADSNAAWAGGFEIWG QGTMTVTSS
4	PR013071 VH FWR1 (Chothia)	EVQLMESGGGLVKPGGSLRLSCVAS
8	PR013071 VH CDR1 (Chothia)	GFTFSDY
12	PR013071 VH FWR2 (Chothia)	YMSWIRQAPGKGLEWVSYI

16	PR013071 VH CDR2 (Chothia)	STSGST
20	PR013071 VH FWR3 (Chothia)	MYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAIYY CAR
81	PR013071 VH CDR3 (Chothia)	KADSNWAGGFEI
27	PR013071 VH FWR4 (Chothia)	WGQGTMTVTVSS
SEQ ID NO:	PR013072	anti-TFR PR012592_N103A HCAb hIgG1(C220S,AAA)
110	PR013072 Heavy Chain	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWIR QAPGKGLEWVSYISTSGSTMYYADSVKGRFTISRDNANK NSLYLQMNSLRAEDTAIYYCARKEDSAWAGGFEIWG QGTMTVTVSSEPKSSDKTHTCPPCPAPEAAGAPSVFLFPP KPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKISKAKGQPREPQVYTLPPSREEMT KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPV LDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
101	PR013072 VH	EVQLMESGGGLVKPGGSLRLSCVASGFTFSDYYMSWIR QAPGKGLEWVSYISTSGSTMYYADSVKGRFTISRDNANK NSLYLQMNSLRAEDTAIYYCARKEDSAWAGGFEIWG QGTMTVTVSS
4	PR013072 VH FWR1 (Chothia)	EVQLMESGGGLVKPGGSLRLSCVAS
8	PR013072 VH CDR1 (Chothia)	GFTFSDY
12	PR013072 VH FWR2 (Chothia)	YMSWIRQAPGKGLEWVSYI
16	PR013072 VH CDR2 (Chothia)	STSGST
20	PR013072 VH FWR3 (Chothia)	MYYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTAIYY CAR
82	PR013072 VH CDR3 (Chothia)	KEDSAWAGGFEI

27	PR013072 VH FWR4 (Chothia)	WGQGTMTVSS
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Example 10: Binding Affinities of PR012592-Derived Mutants to TFR1

10.1 Binding affinity of PR012592 mutants to human and cynomolgus TFR1 proteins

[0367] Binding of recombinant anti-TFR1 antibodies to human TFR1-His protein (Acro, Catalog # CD1-H5243) and cyno TFR1-His protein (Acro, Catalog # TFR-C524a) were tested by ELISA using the procedures as described in Example 5.1.

[0368] The results are shown in Figure 9, Figure 10 and Table 9. The results indicate that the mutants of PR012592 show different binding affinity to human and cyno TFR1-His proteins.

[0369] Table 9. PR012592 mutants binding to human and cyno TFR1-His proteins by ELISA

Antigen	Antibody ID	EC ₅₀ (nM)
human TFR1-His protein	PR013071	8.41
	PR013072	9.09
	PR012838	30.00
	PR012839	30.00
	PR012592	1.92
Cyno TFR1-His protein	PR013071	8.10
	PR013072	11.11
	PR012838	30.00
	PR012839	30.00
	PR012592	2.61

10.2 Binding affinity of PR012592 mutants to TFR1 expressing cell line

[0370] Binding of PR012592 mutants to human TFR1-overexpressing cells HEK293T-cynoTFR1 was tested by flow cytometry using similar procedures as described in Example 5.2.

[0371] The results are shown in Figure 11 and Table 10. These results show that the mutants show different binding affinities to HEK293T-huTFR1 cells.

[0372] Table 10. PR012592 mutants binding to HEK293T-huTFR1 cells by FACS

Target Cell	Antibody ID	EC ₅₀	MFI Max
HEK293T-huTFR1 cells	PR013071	13.69	205081
	PR013072	17.55	197036
	PR012838	9.30	143817
	PR012839	129.20	109079
	PR012592	21.21	266383

MFI Max: calculated maximum MFI.

10.3 Binding affinity of PR012592 mutants to TFR1 proteins by BLI method

[0373] The binding affinities of PR012592 mutants to human and cyno TFR1 proteins were analyzed by BLI using the procedures as described in Example 8. The binding kinetics parameters for anti-TFR1 HCAb antibodies binding to human TFR1 and cyno TFR1 are summarized in Table 11.

[0374] Table 11. Kinetics of anti-TFR1 antibodies to soluble human and cyno TFR1 proteins

Antibody ID	Human TFR1 K _D (M)	Cyno TFR1 K _D (M)
PR013071	<1.0E-12	<1.0E-12
PR013072	6.55E-10	<1.0E-12
PR012838	6.58E-09	3.43E-09
PR012839	9.40E-09	1.37E-08
PR012592	<1.0E-12	<1.0E-12

Example 11: BACE1×TFR1 and Aβ×TFR1 Bispecific Antibodies

[0375] To evaluate the application of anti-TFR1 antibodies for BBB shuttling in the context of bispecific antibodies, BACE1×TFR1 and Aβ×TFR1 bispecific antibodies (bsAb) were produced and evaluated.

[0376] BACE1×TFR1 bsAbs were generated with the format shown in Figure 12, in which the BACE1 binding domain (anti-BACE1 Fab) was derived from sequences of Ab6266 in US11008403B2 (Antibody ID PR013076 in this disclosure), and the TFR1-binding domains (anti-TFR1 VHs) were derived from PR012592 and its mutants. A positive control BACE1×TFR1 bsAb PR013078 was also generated with the sequences of anti-BACE1 Ab6266 and anti-TFR1 Hu15G11.LC92A.M108L disclosed in US11008403B2. A negative control BACE1×Lysozyme bsAb PR013079 was also generated with the sequences of anti-BACE1 Ab6266 and the sequences of cAb-Lys3 nanobody targeting to chicken egg lysozyme. In order to form heterodimeric Fc for BACE1×TFR1 bsAbs, “knobs-into-holes” technology was employed. The “knobs” mutations (S354C, T366W) were introduced to the heavy chain encoding the heavy chain variable region of the anti-BACE1 Fab, and the “holes” mutations (Y349C, T366S, L368A, Y407V) were introduced to the heavy chain encoding anti-TFR1 domain. In order to abolish Fc effector functions, triple mutations **L234A, L235A, G237A** (denoted as “AAA”) were introduced to CH2 of both heavy chains. Same mutations were also applied for BACE1×Lysozyme bsAb. The SEQ ID NOs of amino acid sequences for BACE1×TFR1 and BACE1×Lysozyme bsAbs are listed in Table 12. The SEQ ID NOs of CDR sequences for each antigen-binding fragment in anti-TFR1 HCAb-derived BACE1×TFR1 bsAbs are listed in Table 13. The SEQ ID NOs of amino acid sequences for Ab6266 (PR013076) and cAb-Lys3 (PR000336) are listed in Table 16.

[0377] Aβ×TFR1 bsAbs were generated with the format show in Figure 13, in which the Aβ-binding domains (anti-Aβ Fabs) were derived from sequences of trontinemab (Antibody ID

PR014308 in this disclosure), and the TFR1-binding domains (anti-TFR1 VHs) were derived from PR012592 and its mutants. A positive control A β ×TFR1 bsAb trontinemab (Antibody ID PR012931 in this disclosure) was also generated with published sequences. A negative control A β ×Lysozyme bsAb PR013351 was also generated with the sequences of anti-A β Fab of trontinemab and the sequences of cAb-Lys3 nanobody targeting to chicken egg lysozyme. In order to form heterodimeric Fc for A β ×TFR1 bsAb trontinemab, “knobs-into-holes” technology was employed; and in order to reduce the chain mispairing of different VHs and VLs, some mutations were introduced into the CH1 and CL domains of the anti-A β Fab. In the structure of trontinemab, the anti-TFR1 Fab is appended to the C-terminus of one Fc. When generating A β ×TFR1 bsAbs from anti-TFR1 HCAb PR012592 and its mutants, the anti-TFR1 VH was used to directly replace the anti-TFR1 Fab of trontinemab. The SEQ ID NOs of amino acid sequences for A β ×TFR1 and A β ×Lysozyme bsAbs are listed in Table 14. The SEQ ID NOs of CDR sequences for each antigen-binding fragment in anti-TFR1 HCAb-derived A β ×TFR1 bsAbs are listed in Table 15. The SEQ ID NOs of amino acid sequences for anti-A β Fab of trontinemab (PR014308) and cAb-Lys3 (PR000336) are listed in Table 16.

[0378] Table 12. The SEQ ID NOs of amino acid sequences for BACE1×TFR1 and BACE1×Lysozyme bsAbs

BACE1×TFR1	anti-TFR1	anti-BACE1	Chain-1	Chain-2	Chain-3	Chain-4
PR013078	Hu15G11.LC 92A.M108L	Ab6266	122	113	121	120
PR013356	PR012592	Ab6266	122	113	129	
PR013357	PR012838	Ab6266	122	113	130	
PR013358	PR012839	Ab6266	122	113	131	
PR013359	PR013071	Ab6266	122	113	132	
PR013360	PR013072	Ab6266	122	113	133	
BACE1xLysozyme	anti-Lysozyme	Aanti-BACE1	Chain-1	Chain-2	Chain-3	
PR013079	cAb-Lys3	Ab6266	122	113	123	

[0379] Table 13. The SEQ ID NOs of CDR sequences for each antigen-binding fragment in BACE1×TFR1.

BACE1×TFR1	Antigen binding fragment	LCDR 1	LCDR 2	LCDR 3	HCDR 1	HCDR 2	HCDR 3
PR013356	anti-TFR1				8	16	24
	anti-BACE1	88	91	95	67	73	83
PR013357	anti-TFR1				8	16	79

	anti-BACE1	88	91	95	67	73	83
PR013358	anti-TFR1				8	16	80
	anti-BACE1	88	91	95	67	73	83
PR013359	anti-TFR1				8	16	81
	anti-BACE1	88	91	95	67	73	83
PR013360	anti-TFR1				8	16	82
	anti-BACE1	88	91	95	67	73	83

[0380] Table 14. The SEQ ID NOs of amino acid sequences for A β ×TFR1 and A β ×Lysozyme bsAbs

A β ×TFR1	anti-TFR1	anti-A β	Chain-1	Chain-2	Chain-3	Chain-4
PR012931	trontinemab		118	117	115	116
PR012932	PR012592	PR014308	119	117	115	
PR013352	PR012838	PR014308	125	117	115	
PR013353	PR012839	PR014308	126	117	115	
PR013354	PR013071	PR014308	127	117	115	
PR013355	PR013072	PR014308	128	117	115	
A β ×Lysozyme	anti-Lysozyme	anti-A β	Chain-1	Chain-2	Chain-3	
PR013351	PR000336	PR014308	124	117	115	

[0381] Table 15. The SEQ ID NOs of CDR sequences for each antigen-binding fragment in A β ×TFR1.

A β ×TFR1	Antigen binding fragment	LCDR 1	LCDR 2	LCDR 3	HCDR 1	HCDR 2	HCDR 3
PR012932	anti-TFR1				8	16	24
	anti-A β	89	92	96	68	74	84
PR013352	anti-TFR1				8	16	79
	anti-A β	89	92	96	68	74	84
PR013353	anti-TFR1				8	16	80
	anti-A β	89	92	96	68	74	84
PR013354	anti-TFR1				8	16	81
	anti-A β	89	92	96	68	74	84

PR013355	anti-TFR1				8	16	82
	anti-A β	89	92	96	68	74	84

[0382] Table 16. The SEQ ID NOs of amino acid sequences for anti-BACE1 Ab6266, anti-A β Fab of trontinemab, and anti-lysozyme cAb-Lys3

Antibody ID	Description	VL	VH	LCD R1	LCD R2	LCD R3	HCD R1	HCD R2	HCD R3
PR013076	anti-BACE1 Ab6266 (US11008403B2)	104	102	88	91	95	67	73	83
PR014308	anti-A β Fab of trontinemab	105	103	89	92	96	68	74	84
PR000336	anti lysozyme cAb-Lys3		97				66	72	78

Example 12: Binding Affinities of BsAbs to TFR1

12.1 Binding affinities of BsAbs to human TFR1 protein

[0383] Binding of the BsAbs to human TFR1-His protein (Acro Biosystems, Catalog # CD1-H5243) were tested by ELISA using the procedures as described in Example 5.1.

[0384] The results are shown in Figure 14, Figure 15 and Table 17. The results indicate that the BsAbs show different binding affinities to human TFR1-His protein.

[0385] Table 17. Binding of A β ×TFR1 and BACE1×TFR1 bsAbs to human TFR1-His protein by ELISA

Antigen	Antibody ID	EC ₅₀ (nM)
human TFR1-His protein	PR012932(A β ×PR012592)	0.34
	PR013352(A β ×PR012838)	~58.70
	PR013353(A β ×PR012839)	~33.33
	PR013354(A β ×PR013071)	1.27
	PR013355(A β ×PR013072)	7.73
	PR013351(A β ×Lysozyme)	NA
human TFR1-His protein	PR013356(BACE1×PR012592)	6.03
	PR013357(BACE1×PR012838)	~50.00
	PR013358(BACE1×PR012839)	~33.33
	PR013359(BACE1×PR013071)	11.51
	PR013360(BACE1×PR013072)	36.84

	PR013079(BACE1xLysozyme)	70.51
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12.2 Binding affinity of BsAbs to TFR1 expressing cell lines

[0386] Binding of the BsAbs to human (HEK293T-huTFR1) or cyno (CHOK1-cynoTFR1) TFR1-overexpressing cells was tested by flow cytometry using similar procedures as described in Example 5.2.

[0387] The results are shown in Figure 16, Figure 17, Figure 18 and Table 18. These results show that the BsAbs show different binding affinity to HEK293T-huTFR1 cells and CHOK1-cynoTFR1 cells.

[0388] Table 18. Binding of A β ×TFR1 and BACE1×TFR1 bsAbs to HEK293T-huTFR1 and CHOK1-cynoTFR1 cells by FACS

Target Cell	Antibody ID	EC ₅₀	MFI Max
HEK293T-huTFR1 cells	PR012932	13.14	114908
	PR013352	~40.00	24896
	PR013353	132.60	79406
	PR013354	12.67	71375
	PR013355	48.62	78045
	PR013356	9.83	117812
	PR013357	~40.00	51494
	PR013358	~50.00	9956
	PR013359	32.77	95895
	PR013360	~30.00	64053
CHOK1-cynoTFR1 cells	PR013356	7.04	248981
	PR013357	112	73138
	PR013358	NA	12161
	PR013359	10.09	154164
	PR013360	11.03	153252

MFI Max: calculated maximum MFI.

Example 13: *In vivo* Evaluation of BACE1×TFR1 BsAbs

[0389] Human TFR1 knock-in (hTFR1-KI) transgenic mice (Biocytogen) were used to evaluate the efficacy of BACE1×TFR1 BsAbs *in vivo*. The experimental design is shown in Figure 19 and Table 19. For 6 mice in each group, 3 mice brains and sera were taken at 24 hours or 48 hours after administration, and half of the brains were homogenized and subjected

to capillary depletion, and the concentration of drugs entering the brain was determined by ELISA. The other half of the brain was paraffin-embedded and sectioned, and the distribution of the drug in the brain was detected by Immunohistochemistry (IHC).

[0390] IHC detection of Human IgG: (1) Dewaxing and hydration: tissue slices were dewaxed in fixed order in xylene I, xylene II, and xylene III, each for 10 minutes, washed in anhydrous ethanol I and anhydrous ethanol II sequentially for 5 minutes and then sequentially hydrated in 95% ethanol, 70% ethanol, and pure water for 5 minutes. (2) Antigen retrieval: tissue slices were placed into boiled antigen retrieval solution and subjected to antigen retrieval with low heat in a microwave and then cooled to room temperature; then the slices were washed 3 times with PBS (3 minutes each time), sealed with 3% hydrogen peroxide at room temperature for 10 minutes, washed 3 times with PBS (3 minutes each time) and blocked with antigen blocking solution at 37 °C for 30 minutes. (3) Secondary antibody incubation: slices were incubated with the secondary antibody (Jackson Immuno Research, 109-036-098) at 37 °C for 30 minutes; after washing with PBS, the slices were washed 3 times with PBS (3 minutes each time) and incubated with ABC reagent at 37 °C for 30 minutes; after washing with PBS, the slices were washed 3 times with PBS (3 minutes each time). (4) Substrate colorimetry: the slices were incubated with substrate colorimetric solution at room temperature for 3-10 minutes, then washed with PBS to stop color development and stained with hematoxylin for 15-60 seconds and then washed with PBS and rinsed under tap water for 3 minutes. (5) Sealing: the slices were washed sequentially with 95% ethanol, anhydrous ethanol III, and anhydrous ethanol IV for 2 minutes, and then washed with anhydrous ethanol: xylene=1:1, anhydrous ethanol IV, and anhydrous ethanol V for 1 minute and sealed with neutral resin.

[0391] Table 19. Study Design.

Group #	BACE1×TFR1 bsAb	Mice	No.	Dose	Time	Readout
G1	PR013079	hTFR1 KI mouse	6	10 mg/kg	24 h, 48 h	Immunohistochemistry , Brain and parenchyma ELISA
G2	PR013078					
G3	PR013356					
G4	PR013357					
G5	PR013358					
G6	PR013359					
G7	PR013360					

[0392] The results of ELISA are shown in Figures 20, 21 and 22. As shown in Figures 20 and 21, the BACE1×TFR1 bsAbs exhibited differential brain-penetrating abilities. Surprisingly, anti-TFR1 HCAb-derived BACE1×TFR1 bsAbs PR013357, PR013359 and PR013360 demonstrated comparable or even stronger brain penetration than the positive control PR013078.

[0393] The results of IHC are shown in Figure 23, in which, arrows point to neurons uptaking the BsAbs. Consistent with the ELISA results, the BACE1×TFR1 bsAbs exhibited differential brain uptake and distribution. Surprisingly, anti-TFR1 HCAb-derived BACE1×TFR1 bsAbs PR013357, PR013359 and PR013360 demonstrated comparable or even more neuron uptake and brain parenchyma distribution than the positive control PR013078.

[0394] It is to be understood that the foregoing description of the embodiments is intended to be purely illustrative of the principles of the disclosure, rather than exhaustive thereof, and that changes and variations will be apparent to those skilled in the art, and that the present disclosure is not intended to be limited other than expressly set forth in the following claims.

Claims

1. An anti-transferrin receptor 1 (TFR1) antibody or an antigen-binding fragment thereof, comprising a heavy chain variable domain, wherein the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3, wherein

the VH CDR1 comprises an amino acid sequence differing from SEQ ID NO: 8 by addition, deletion or substitution of no more than 2 amino acids;

the VH CDR2 comprises an amino acid sequence differing from SEQ ID NO: 16 by addition, deletion or substitution of no more than 2 amino acids; and/or

the VH CDR3 comprises an amino acid sequence differing from SEQ ID NO: 24 by addition, deletion or substitution of no more than 2 amino acids.

2. The anti-TFR1 antibody or antigen-binding fragment of claim 1, wherein
the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8,
the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and
the VH CDR3 comprises an amino acid sequence differing from SEQ ID NO: 24 by amino acid addition, deletion or substitution of no more than 2 amino acids;

preferably, the VH CDR3 comprises a non-conservative amino acid substitution at a position corresponding to amino acid 2, 4, 5, or 7 of SEQ ID NO: 24.

3. The anti-TFR1 antibody or antigen-binding fragment of claim 1 or 2, wherein
the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 8,
the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 16, and
the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 24, 79, 80, 81 or 82.

4. The anti-TFR1 antibody or antigen-binding fragment of claim 1, wherein the heavy chain variable domain comprises a VH CDR1, a VH CDR2 and a VH CDR3 as comprised in the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101.

5. The anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-4, wherein the heavy chain variable domain comprises:

(A) the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101; or

(B) an amino acid sequence that is at least 80%, 85%, 90%, 95%, 98% or 99% identical to the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101; or

(C) an amino acid sequence having amino acid addition, deletion and/or substitution of one or more amino acids in comparison with the amino acid sequence of SEQ ID NO: 52, 98, 99, 100 or 101, wherein preferably, the amino acid addition, deletion and/or substitution do/does not occur in a CDR region.

6. The anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-5, which
- (1) binds to human TFR1 and non-human primate TFR1; and/or
 - (2) does not substantially affect the binding of transferrin to TFR1; and/or
 - (3) binds to the transferrin-TFR1 complex.

7. The anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-6, which is a single domain antibody or a heavy-chain-only antibody and optionally a fully human antibody.

8. The anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-7, further comprising an Fc region, and optionally the Fc region comprises one or more modifications to reduce the effector function and/or enhance the FcRn binding.

9. The anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-8, comprising a heavy chain having the amino acid sequence of SEQ ID NO: 59, 107, 108, 109 or 110.

10. A transferrin receptor 1 (TFR1)-binding agent comprising the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 conjugated to an additional molecule.

11. The TFR1-binding agent of claim 10, wherein the additional molecule is a therapeutic agent for treating a disease or disorder selected from: cancer, central nervous system cancer, Alzheimer's disease, Parkinson's disease, Huntington's disease, hunter syndrome, schizophrenia, antidepressant, multiple sclerosis, amyotrophic lateral sclerosis, lysosomal storage disease accompanied by encephalopathy, glycogenosis, muscular dystrophy, cerebral ischemia, a prion disease, traumatic central nervous system disorder, viral and bacterial central nervous system disease.

12. The TFR1-binding agent of claim 10, wherein the additional molecule is an imaging agent selected from: a radionuclide, biotin, a fluorescent protein, a fluorophore, horseradish peroxidase and alkaline phosphatase.

13. A multispecific antibody, comprising a first antigen-binding moiety binding to TFR1, wherein the first antigen-binding moiety comprises the heavy chain variable domain as defined in any one of claims 1-6, and a second antigen-binding moiety binding to a second antigen.

14. A multispecific antibody, comprising a first antigen-binding moiety binding to TFR1 and a second antigen-binding moiety binding to BACE1, wherein

the first antigen-binding moiety comprises the heavy chain variable domain as defined in any one of claims 1-6,

the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HCDR1, an HCDR2 and an HCDR3 as comprised in the amino acid sequence of SEQ ID NO: 102, and the light chain variable region comprises an LCDR1, an LCDR2 and an LCDR3 as comprised in the amino acid sequence of SEQ ID NO: 104;

preferably, the HCDR1 comprises an amino acid sequence of SEQ ID NO: 67, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 73, and the HCDR3 comprises an amino acid sequence of SEQ ID NO: 83; and the LCDR1 comprises an amino acid sequence of SEQ ID NO: 88, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 91, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 95.

15. The multispecific antibody of claim 14, wherein

the heavy chain variable region comprises an amino acid sequence of SEQ ID NO: 102; and/or the light chain variable region comprises an amino acid sequence of SEQ ID NO: 104.

16. A multispecific antibody, comprising a first antigen-binding moiety binding to TFR1 and a second antigen-binding moiety binding to amyloid beta (A β), wherein

the first antigen-binding moiety comprises the heavy chain variable domain as defined in any one of claims 1-6,

the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises an HCDR1, an HCDR2 and an HCDR3 as comprised in the amino acid sequence of SEQ ID NO: 103, and the light chain variable region comprises an LCDR1, an LCDR2 and an LCDR3 as comprised in the amino acid sequence of SEQ ID NO: 105;

preferably, the HCDR1 comprises an amino acid sequence of SEQ ID NO: 68, the HCDR2 comprises an amino acid sequence of SEQ ID NO: 74, and the HCDR3 comprises an amino acid sequence of SEQ ID NO: 84; and the LCDR1 comprises an amino acid sequence of SEQ ID NO: 89, the LCDR2 comprises an amino acid sequence of SEQ ID NO: 92, and the LCDR3 comprises an amino acid sequence of SEQ ID NO: 96.

17. The multispecific antibody of claim 16, wherein

the heavy chain variable region comprises an amino acid sequence of SEQ ID NO: 103; and/or

the light chain variable region comprises an amino acid sequence of SEQ ID NO: 105.

18. The multispecific antibody of any one of claims 13-17, wherein the second antigen-binding moiety comprises a dsFv, an scFv, an scdsFv, a di-scFv, an Fab, an scFab or an F(ab')₂.

19. The multispecific antibody of any one of claims 13-18, which comprises only one heavy chain variable domain as defined in any one of claims 1-6.

20. The multispecific antibody of any one of claims 13-19, further comprising an Fc region, and optionally the Fc region comprises one or more modifications to reduce the effector function and/or enhance the FcRn binding.

21. The multispecific antibody of claim 20, wherein the Fc region is a heterodimeric Fc region comprising a first Fc region subunit and a second Fc region subunit, wherein one of the first Fc region subunit and the second Fc region subunit comprises a knob mutation, and the other comprises a hole mutation.

22. The multispecific antibody of claim 21, wherein
the knob mutation comprises one or more mutations selected from S354C and T366W;
and/or

the hole mutation comprises one or more mutations selected from Y349C, T366S, L368A and Y407V;

preferably, the knob mutation comprises S354C and T366W, and the hole mutation comprises Y349C, T366S, L368A and Y407V.

23. The multispecific antibody of claim 21 or 22, wherein the second antigen-binding moiety comprises a heavy chain variable region and a light chain variable region, and the multispecific antibody comprises:

(a) (1) a first polypeptide comprising the heavy chain variable region, a CH1 domain and a first Fc region subunit, (2) a second polypeptide comprising the light chain variable region and a light chain constant region, and (3) a third polypeptide comprising the heavy chain variable domain and a second Fc region subunit, or

(b) (1) a first polypeptide comprising the heavy chain variable region, a CH1 domain, a first Fc region subunit, an optional peptide linker and the heavy chain variable domain, (2) a second polypeptide comprising the heavy chain variable region, a CH1 domain and a second Fc region subunit, and (3) a third polypeptide and a fourth polypeptide each comprising the light chain variable region and a light chain constant region,

wherein the first Fc region subunit and the second Fc region subunit form the heterodimeric Fc region.

24. The multispecific antibody of claim 14, comprising a first polypeptide having the amino acid sequence of SEQ ID NO: 122, a second polypeptide having the amino acid sequence of SEQ ID NO: 113, and a third polypeptide having the amino acid sequence of SEQ ID NO: 129, 130, 131, 132 or 133.

25. The multispecific antibody of claim 16, comprising a first polypeptide having the amino acid sequence of SEQ ID NO: 119, 125, 126, 127 or 128, a second polypeptide having the amino acid sequence of SEQ ID NO: 117, and a third polypeptide and a fourth polypeptide each having the amino acid sequence of SEQ ID NO: 115.

26. A pharmaceutical composition or a kit comprising the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9, the TFR1-binding agent of any one of claims 10-12 or the multispecific antibody of any one of claims 13-25.

27. A nucleic acid encoding the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9, or the multispecific antibody of any one of claims 13-25.

28. A vector comprising the nucleic acid of claim 27.

29. A host cell, comprising the nucleic acid of claim 27 or the vector of claim 28.

30. Use of the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 in the manufacture of an agent for transporting a compound across the blood-brain barrier.

31. Use of the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 in the manufacture of a medicament for treating cancer.

32. Use of the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 in the manufacture of a medicament for treating a neurological disease or disorder, wherein the anti-TFR1 antibody or antigen-binding fragment is conjugated to a therapeutic agent for treating the neurological disease or disorder.

33. Use of the multispecific antibody of any one of claims 14-25 in the manufacture of a medicament for treating a neurological disease or disorder; preferably, the neurological disease or disorder is selected from Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury and glaucoma.

34. Use of the multispecific antibody of any one of claims 14-25 in the manufacture of an agent or a kit for diagnosing Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury or glaucoma.

35. Use of the multispecific antibody of any one of claims 14-25 in the manufacture of a medicament for inhibiting and/or decreasing the formation of amyloid plaques in the brain.

36. A method of transporting a compound across the blood-brain barrier in a subject, comprising conjugating the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 to the compound and transporting the conjugate across the blood-brain barrier.

37. A method of treating cancer in a subject in need thereof, comprising administering a therapeutically effective amount of the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 to the subject.

38. A method of treating a neurological disease or disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 conjugated to a therapeutic agent for treating the neurological disease or disorder to the subject.

39. A method of treating a neurological disease or disorder in a subject in need thereof, comprising administering a therapeutically effective amount of the multispecific antibody of any one of claims 14-25 to the subject; preferably, wherein the neurological disease or disorder is selected from Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury and glaucoma.

40. A method of inhibiting and/or decreasing the formation of amyloid plaques in the brain of a subject in need thereof, comprising administering a therapeutically effective amount of the multispecific antibody of any one of claims 14-25 to the subject to inhibit and/or decrease the formation of amyloid plaques in the brain.

41. The anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 for use as a medicament, wherein the anti-TFR1 antibody or antigen-binding fragment is transporting a compound across the blood-brain barrier.

42. The anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 for use in treating cancer.

43. The anti-TFR1 antibody or antigen-binding fragment of any one of claims 1-9 for use as a medicament for treating a neurological disease or disorder, wherein the anti-TFR1 antibody or antigen-binding fragment is conjugated to a therapeutic agent for treating the neurological disease or disorder.

44. The multispecific antibody of any one of claims 14-25 for use in treating a neurological disease or disorder, optionally, wherein the neurological disease or disorder is selected from

Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury and glaucoma.

45. The multispecific antibody of any one of claims 14-25 for use in diagnosing Alzheimer's disease, Parkinson's disease, spinal cord injury, Amyotrophic Lateral Sclerosis (ALS), stroke, traumatic brain injury or glaucoma.

46. The multispecific antibody of any one of claims 14-25 for use in inhibiting and/or decreasing the formation of amyloid plaques in the brain.