One of ordinary skill in the art will appreciate that any combination of the foregoing methods may be employed to engineer an antibody (and/or dosage regimen for same) with the optimum balance between (i) the desirably low affinity for primate or human TfR that will maximize transport of the antibody and any conjugated compounds into the CNS; (ii) the affinity of the conjugated compound (including as a nonlimiting example, a second or further antigen-binding specificity in the anti-human/cyno TfR antibody) for its CNS antigen, since this is relevant to the amount of the compound that needs to be present in the CNS to have a therapeutic effect; (iii) the clearance rate of the anti-human/cynoTfR antibody; (iv) the lability of the anti-TfR/conjugated compound at low pH to facilitate release of the conjugated compound on the CNS/brain side of the BBB, and (v) the impact on reticulocyte populations.

It will also be appreciated that the reticulocyte-depleting effect recognized herein of anti-TfR antibody administration may be useful in the treatment of any disease or disorder where overproliferation of reticulocytes is problematic. For example, in congenital polycythemia or neoplastic polycythemia vera, raised red blood cell counts due to hyperproliferation of, e.g., reticulocytes, results in thickening of blood and concomitant physiological symptoms. Administration of an anti-human/cyno TfR antibody of the disclosure wherein at least partial effector function of the antibody was preserved would permit selective removal of immature reticulocyte populations without impacting normal transferrin transport into the CNS. Dosing of such an antibody could be modulated such that acute clinical symptoms could be minimized (ie, by dosing at a very low dose or at widely-spaced intervals), as well-understood in the art.

Anti-TfR/BACE1 and anti-TfR/Abeta are each promising and novel therapeutic candidates for the treatment of Alzheimer's disease. Furthermore, receptor mediated transport (RMT)-based bispecific targeting technology opens the door for a wide range of potential therapeutics for CNS diseases. The disclosure provides methods of engineering BBB-penetrant therapeutics that greatly improve transport across the BBB and CNS distribution of the therapeutic without depletion of reticulocytes.

In a first aspect, there is provided an isolated antibody that binds to human transferrin receptor (TfR) and non-human primate TfR, wherein the antibody does not inhibit the binding of transferrin to TfR, wherein the antibody comprises HVR-H1, HVR-H2, and HVR-H3, respectively comprising the amino acid sequences of SEQ ID NOs: 53, 156 and 55; and comprises HVR-L1, HVR-L2, and HVR-L3, respectively comprising the amino acid sequences of SEQ ID NOs: 50, 51 and 52. In one embodiment, the binding is specific binding. In another embodiment, the antibody further does not inhibit the binding of human hemachromatosis protein ("HFE") to TfR. In a second aspect, there is provided an isolated antibody that binds to human TfR and non-human primate TfR, wherein the antibody does not inhibit the binding of transferrin to TfR, and wherein one or more properties of the antibody have been modified to reduce or eliminate the impact of the antibody on reticulocytes and/or reduce the severity or presence of acute clinical symptoms in a subject or mammal treated with the antibody, wherein the one or more properties are selected from the effector function of the antibody Fc region and the complement activation function of the antibody, and wherein the effector function or complement activation function has been reduced or eliminated relative to a wild-type antibody of the same isotype, and wherein the antibody comprises HVR-H1, HVR-H2, and HVR-H3, respectively comprising the amino acid sequences of SEQ ID NOs: 53, 156 and 55; and comprises HVR-L1, HVR-L2, and HVR-L3, respectively comprising the amino acid sequences of SEQ ID NOs: 50, 51 and 52. In one embodiment, the binding is specific binding. In one embodiment, the antibody is a monoclonal antibody. In another embodiment, the antibody is a human antibody. In another embodiment, the antibody is a humanized antibody. In another embodiment, the antibody is a

16F6.v2, 16F6.v3 and 16F6.v4. In one such embodiment, the antibody is 15G11.v1. In another such embodiment, the antibody is 15G11.v2. In another such embodiment, the antibody is 15G11.v3. In another such embodiment, the antibody is 15G11.v4. In another such embodiment, the antibody is 15G11.v5. In another such embodiment, the antibody is 7A4.v1. In another such embodiment, the antibody is 7A4.v2. In another such embodiment, the antibody is 7A4.v3. In another such embodiment, the antibody is 7A4.v4. In another such embodiment, the antibody is 7A4.v5. In another such embodiment, the antibody is 7A4.v6. In another such embodiment, the antibody is 7A4.v7. In another such embodiment, the antibody is 7A4.v8. In another such embodiment, the antibody is 7A4.v9. In another such embodiment, the antibody is 7A4.v10. In another such embodiment, the antibody is 7A4.v11. In another such embodiment, the antibody is 7A4.v12. In another such embodiment, the antibody is 7A4.v13. In another such embodiment, the antibody is 7A4.v14. In another such embodiment, the antibody is 7A4.v15. In another such embodiment, the antibody is 7G7.v1. In another such embodiment, the antibody is 16F6.v1. In another such embodiment, the antibody is 16F6.v2. In another such embodiment, the antibody is 16F6.v3. In another such embodiment, the antibody is 16F6.v4. In another such embodiment, the antibody is 15G11.N52A. In another such embodiment, the antibody is 16F6.v4. In another such embodiment, the antibody is 15G11.T53A. In another such embodiment, the antibody is 16F6.v4. In another such embodiment, the antibody is 15G11.W92A. In another such embodiment, the antibody is 16F6.v4.

Disclosed is an isolated antibody that binds to the same epitope on TfR as the antibody of the third aspect. In one embodiment, the antibody is modified at one or more amino acid positions in the VH or VL to the amino acid indicated for that position in Figures 4E-1 and 4E-2. In another embodiment, the antibody comprises a sequence or one or more HVR sequences corresponding to that of or one or more of those set forth for any one of the clones in Figures 3 and 4 and Figures 4E-1 and 4E-2. In another embodiment, the antibody comprises a VH or VL sequence corresponding to that of set forth for any one of the clones in Figures 3 and 4 and Figures 4E-1 and 4E-2. In another embodiment, the antibody comprises one or more HVR sequences corresponding to one or more of those set forth for any one of the clones in Figures 3 and 4 and Figures 4E-1 and 4E-2.

In one embodiment, the one or more properties of the antibody are selected from the effector function of the antibody Fc region, the complement activation function of the antibody and the affinity of the antibody for TfR. In one such embodiment, the property is the effector function of the antibody Fc region. In another such embodiment, the property is the complement activation function of the antibody. In another such embodiment, the property is the affinity of the antibody for TfR. In one such embodiment, the effector function or complement

precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6. In another such embodiment, the multispecific antibody binds both TfR and BACE1. In another such embodiment, the multispecific antibody binds both TfR and Abeta. In another such embodiment, the therapeutic compound is a neurological disorder drug.

In a fourth aspect, there is provided an isolated nucleic acid encoding any of the foregoing antibodies. In a fifth aspect, there is provided a host cell comprising the nucleic acid described herein. In a sixth aspect, there is provided a method of producing an antibody comprising culturing the host cell described herein so that the antibody is produced and optionally further comprising recovering the antibody from the host cell. In another embodiment, the disclosure provides a host cell comprising such nucleic acid. In another embodiment, the disclosure provides a method of producing any of the foregoing antibodies comprising culturing such host cell so that the antibody is produced and optionally further comprising recovering the antibody from the host cell.

In a seventh aspect, there is provided a pharmaceutical formulation comprising any of the foregoing antibodies and a pharmaceutically acceptable carrier.

In an eighth aspect, there is provided any of the foregoing antibodies for use as a medicament. In a ninth aspect, there isprovided the use of any of the foregoing antibodies in the manufacture of a medicament for the treatment of a neurological disorder. In one such embodiment, the neurological disorder is selected from the group consisting of a neuropathy disorder, a neurodegenerative disease, cancer, an ocular disease disorder, a seizure disorder, a lysosomal storage disease, amyloidosis, a viral or microbial disease, ischemia, a behavioral disorder, and CNS inflammation.

In a tenth aspect, there is provided any of the foregoing antibodies for use in the treatment of a neurological disorder. In one such embodiment, the neurological disorder is selected from the group consisting of a neuropathy disorder, a neurodegenerative disease, cancer, an ocular disease disorder, a seizure disorder, a lysosomal storage disease, amyloidosis, a viral or microbial disease, ischemia, a behavioral disorder, and CNS inflammation.

In an eleventh aspect, there is provided any of the foregoing antibodies for use in transporting one or more compounds across the BBB. Also disclosed is use of any of the foregoing antibodies in the manufacture of a medicament for transporting one or more compounds across the BBB is provided.

Disclosed is an antibody described herein for use in transporting a compound across the BBB in a subject, wherein when the antibody is exposed to the BBB, the antibody transports the compound coupled thereto across the BBB. In one embodiment, a method of transporting a compound across the BBB in a subject is provided, comprising exposing any of the foregoing antibodies to the BBB such that the antibody transports the compound coupled thereto across the BBB. In another such embodiment, the BBB is in a human subject. In another such embodiment, the dose amount and/or frequency of administration is modulated to reduce the concentration of antibody to

which red blood cells are exposed. In another such embodiment, the method further comprises the step of monitoring the subject for depletion of red blood cells. In another such embodiment, the antibody coupled to the compound is administered at a therapeutic dose. In one such embodiment, the therapeutic dose is TfR-saturating. In another such embodiment, administration of the antibody is at a dose and/or dose frequency calibrated to minimize acute clinical symptoms of the antibody administration.

Disclosed is an antibody described herein for use in increasing exposure of the CNS of a subject to a compound, wherein when the antibody is exposed to the BBB, the antibody transports the compound coupled thereto across the BBB. In another embodiment, a method of increasing exposure of the CNS of a subject to a compound is provided, comprising exposing any of the foregoing antibodies to the BBB such that the antibody transports the compound coupled thereto across the BBB. In another such embodiment, the BBB is in a human subject. In another such embodiment, the dose amount and/or frequency of administration is modulated to reduce the concentration of antibody to which red blood cells are exposed. In another such embodiment, the method further comprises the step of monitoring the subject for depletion of red blood cells. In another such embodiment, the antibody coupled to the compound is administered at a therapeutic dose. In one such embodiment, the therapeutic dose is TfR-saturating. In another such embodiment, administration of the antibody is at a dose and/or dose frequency calibrated to minimize acute clinical symptoms of the antibody administration.

Disclosed an antibody described herein for use in increasing retention in the CNS of a compound administered to a subject, wherein when the antibody is exposed to the BBB, the retention in the CNS of the compound is increased. In one embodiment, a method of increasing rentention in the CNS of a compound administered to a subject is provided, comprising exposing any of the foregoing antibodies to the BBB such that the retention in the CNS of the compound is increased. In another such embodiment, the BBB is in a human subject. In another such embodiment, the dose amount and/or frequency of administration is modulated to reduce the concentration of antibody to which red blood cells are exposed. In another such embodiment, the method further comprises the step of monitoring the subject for depletion of red blood cells. In another such embodiment, the antibody coupled to the compound is administered at a therapeutic dose. In one such embodiment, the therapeutic dose is TfR-saturating. In another such embodiment, administration of the antibody is at a dose and/or dose frequency calibrated to minimize acute clinical symptoms of the antibody administration.

In a twelfth aspect, there is provided use of an antibody described herein in the manufacture of a medicament for treating a neurological disorder in a mammal. In one embodiment, a method of treating a neurological disorder in a mammal is provided, comprising treating the mammal with any of the foregoing antibodies. In one such embodiment, the neurological disorder is selected from the group consisting of a neuropathy disorder, a neurodegenerative disease, cancer, an ocular disease disorder, a seizure disorder, a lysosomal storage disease, amyloidosis, a viral or microbial disease, ischemia, a behavioral disorder, and CNS inflammation. In another such embodiment, the neurological disorder

WHAT IS CLAIMED IS:

1. An isolated antibody that binds to human transferrin receptor (TfR) and non-human primate TfR, wherein the antibody does not inhibit the binding of transferrin to TfR, wherein the antibody comprises HVR-H1, HVR-H2, and HVR-H3, respectively comprising the amino acid sequences of SEQ ID NOs: 53, 156 and 55; and comprises HVR-L1, HVR-L2, and HVR-L3, respectively comprising the amino acid sequences of SEQ ID NOs: 50, 51 and 52.

2. An isolated antibody that binds to human TfR and non-human primate TfR, wherein the antibody does not inhibit the binding of transferrin to TfR, and wherein one or more properties of the antibody have been modified to reduce or eliminate the impact of the antibody on reticulocytes and/or reduce the severity or presence of acute clinical symptoms in a subject or mammal treated with the antibody, wherein the one or more properties are selected from the effector function of the antibody Fc region and the complement activation function of the antibody, and wherein the effector function or complement activation function has been reduced or eliminated relative to a wild-type antibody of the same isotype, and wherein the antibody comprises HVR-H1, HVR-H2, and HVR-H3, respectively comprising the amino acid sequences of SEQ ID NOs: 53, 156 and 55; and comprises HVR-L1, HVR-L2, and HVR-L3, respectively comprising the amino acid sequences of SEQ ID NOs: 50, 51 and 52.

3. The antibody of claim 1 or 2, which is a monoclonal antibody.

4. The antibody of claim 1 or 2, which is a human, humanized, or chimeric antibody.

5. The antibody of claim 1 or 2, which is an antibody fragment that binds human TfR and primate TfR.

6. The antibody of claim 1 or 2, comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 153; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 105 or (c) a VH sequence as in (a) and a VL sequence as in (b).

7. The antibody of claim 6, comprising a VH sequence of SEQ ID NO: 153.

8. The antibody of claim 6, comprising a VL sequence of SEQ ID NO: 105.

9. An antibody comprising a VH sequence of SEQ ID NO: 153 and a VL sequence of SEQ ID NO: 105.

10. The antibody of claim 2, wherein the effector function is reduced or eliminated by a method selected from reduction of glycosylation of the antibody, modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function, and modification of the Fc region.

11. The antibody of claim 10, wherein the glycosylation of the antibody is reduced by a method selected from: production of the antibody in an environment that does not permit wild-type glycosylation; removal of carbohydrate groups already present on the antibody; and modification of the antibody such that wild-type glycosylation does not occur.

12. The antibody of claim 11, wherein the antibody is produced in a non-mammalian cell production system, or where the antibody is produced synthetically.

13. The antibody of claim 11, wherein the Fc region of the antibody comprises a mutation at position 297 such that the wild-type asparagine residue at that position is replaced with another amino acid that interferes with glycosylation at that position.

14. The antibody of claim 10, wherein the effector function is reduced or eliminated by at least one modification of the Fc region.

15. The antibody of claim 14, wherein the effector function or complement activation function is reduced or eliminated by deletion of all or a portion of the Fc region, or by engineering

the antibody such that it does not include an Fc region or non-Fc region competent for effector function or complement activation function.

16. The antibody of claim 14, wherein the modification is selected from: a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 234, 235, 238, 239, 248, 249, 252, 254, 265, 268, 269, 270, 272, 278, 289, 292, 293, 294, 295, 296, 297, 298, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, and 439; a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321; eliminating some or all of the Fc region, and a point mutation at position 132 of the CH1 domain.

17. The antibody of claim 16, wherein the modification is at least one point mutation of the Fc region to impair binding to one or more Fc receptors selected from 234, 235, 265, 297 and 329.

18. The antibody of claim 17, wherein the modification is at positions 297 or 265 and 297.

19. The antibody of claim 17, wherein the modification is at positions 234, 235 and 329.

20. The antibody of claim 18, wherein the modification is N297G; D265A and N297A or D265A and N297G.

21. The antibody of claim 18, wherein the modification is L234A, L235A, and P329G.

22. The antibody of any one of the preceding claims, wherein the half-life of the antibody is increased by a modification in the FcRn binding domain of the antibody at a position selected from: 252, 254, 256, 434 and 436.

23. The antibody of claim 22, wherein the modification is at positions 252, 254 and 256.

24. The antibody of claim 22, wherein the modification is at positions 434 and 436.

25. The antibody of claim 23, wherein the modification is M252Y, S254T and T256E.

26. The antibody of claim 24, wherein the modification is N434A and Y436I.

27. The antibody of claim 2, wherein the antibody is combined with a further compound that mitigates or contributes to the reduction of impact on reticulocyte levels or acute clinical symptoms.

28. The antibody of claim 27, wherein the further compound protects reticulocytes from antibody-related depletion or supports the growth, development, or reestablishment of reticulocytes.

29. The antibody of claim 27, wherein the further compound is selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid, and vitamin B12, or is red blood cells or reticulocytes.

30. The antibody of claim 1 or 2, wherein the antibody has a KD or IC50 for TfR of about 20 nM to about 100 μM.

31. The antibody of claim 1 or 2, wherein the antibody is coupled to a therapeutic compound.

32. The antibody of claim 31, wherein the antibody is a multispecific antibody and the therapeutic compound optionally forms one portion of the multispecific antibody.

33. The antibody of claim 32, wherein the multispecific antibody comprises a first antigen binding site which binds TfR and a second antigen binding site which binds a brain antigen.

34. The antibody of claim 33, wherein the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E (ApoE), alpha-synuclein, CD20,

huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6.

35. The antibody of claim 34, wherein the multispecific antibody binds both TfR and BACE1.

36. The antibody of claim 34, wherein the multispecific antibody binds both TfR and Abeta.

37. The antibody of claim 31, wherein the therapeutic compound is a neurological disorder drug.

38. An isolated nucleic acid encoding the antibody of any of claims 1 to 37.

39. A host cell comprising the nucleic acid of claim 38.

40. A method of producing an antibody comprising culturing the host cell of claim 39 so that the antibody is produced and optionally further comprising recovering the antibody from the host cell.

41. A pharmaceutical formulation comprising the antibody of any of claims 1-37 and a pharmaceutically acceptable carrier.

42. The antibody of any of claims 1-37 for use as a medicament.

43. The antibody of any of claims 1-37 for use in the treatment of a neurological disorder.

44. The antibody of claim 43, wherein the neurological disorder is selected from the group consisting of a neuropathy disorder, a neurodegenerative disease, cancer, an ocular disease disorder, a seizure disorder, a lysosomal storage disease, amyloidosis, a viral or microbial disease, ischemia, a behavioral disorder, and CNS inflammation.

45. The antibody of any of claims 1-37 for use in transporting one or more compounds across the BBB.

46. Use of the antibody of any of claims 1-37 in the manufacture of a medicament for the treatment of a neurological disorder.

47. The use of claim 46, wherein the neurological disorder is selected from the group consisting of a neuropathy disorder, a neurodegenerative disease, cancer, an ocular disease disorder, a seizure disorder, a lysosomal storage disease, amyloidosis, a viral or microbial disease, ischemia, a behavioral disorder, and CNS inflammation.

48. Use of an antibody of any one of claims 31-37 in the manufacture of a medicament for treating a neurological disorder in a mammal.

49. The use of claim 48, wherein the neurological disorder is selected from the group consisting of a neuropathy disorder, a neurodegenerative disease, cancer, an ocular disease disorder, a seizure disorder, a lysosomal storage disease, amyloidosis, a viral or microbial disease, ischemia, a behavioral disorder, and CNS inflammation.

50. The use of claim 48 or 49, wherein the neurological disorder is in a human subject.

51. The use of claim 50, wherein the dose amount and/or frequency of administration is modulated to reduce the concentration of antibody to which the red blood cells are exposed.

52. The use of claim 50, further comprising the step of monitoring the subject for depletion of red blood cells.

53. The use of claim 50, wherein the antibody coupled to the therapeutic compound is to be administered at a therapeutic dose.

54. The use of claim 53, wherein the therapeutic dose is TfR-saturating.

55. The use of claim 50, wherein administration of the antibody is at a dose and/or dose frequency calibrated to minimize acute clinical symptoms of the antibody administration.

56. The antibody for use according to claim 45, wherein the BBB is in a human subject.

57. The antibody for use according to claim 56, wherein the dose amount and/or frequency of administration is modulated to reduce the concentration of antibody to which the red blood cells are exposed.

58. The antibody for use according to claim 56, further comprising the step of monitoring the subject for depletion of red blood cells.

59. The antibody for use according to claim 56, wherein the antibody coupled to the compound is to be administered at a therapeutic dose.

60. The antibody for use according to claim 59, wherein the therapeutic dose is TfR-saturating.

61. The antibody for use according to claim 56, wherein administration of the antibody is at a dose and/or dose frequency calibrated to minimize acute clinical symptoms of the antibody administration.