LOW AFFINITY BLOOD BRAIN BARRIER RECEPTOR ANTIBODIES AND USES THEREFOR

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 61/418,223 filed on 30 November 2010, which is incorporated by reference in its entirety.

FIELD OF THE INVENTION

The present invention relates to antibodies that bind blood brain barrier receptors (BBB-R) and methods of using the same.

BACKGROUND

Brain penetration of large molecule drugs is severely limited by the largely impermeable blood-brain barrier (BBB). Among the many strategies to overcome this obstacle is to utilize transcytosis trafficking pathways of endogenous receptors expressed at the brain capillary endothelium. Recombinant proteins such as monoclonal antibodies have been designed against these receptors to enable receptor-mediated delivery of large molecules to the brain. However, strategies to maximize brain uptake while minimizing reverse transcytosis back to the blood, and the extent of accumulation after therapeutic dosing, remain unexplored. Furthermore, whether antibodies that cross the BBB are pharmacodynamically functional is unknown.

SUMMARY

Monoclonal antibodies have vast therapeutic potential for treatment of neurological or central nervous system (CNS) diseases, but their passage into the brain is restricted by the blood-brain barrier (BBB). Past studies have shown that a very small percentage (approximately 0.1%) of an IgG circulating in the bloodstream crosses through the BBB into the CNS (Felgenhauer, Klin. Wschr. 52: 1158-1164 (1974)), where the CNS concentration of the antibody may be insufficient to permit a robust effect. The methods and compositions of the ~~invention~~disclosure provide a way to improve the percentage of the antibody that distributes into the CNS and thus more readily attain therapeutic antibody concentrations in the CNS.

Herein is described a group of antibodies against the transferrin receptor (TfR) that can deliver therapeutics including antibodies and small molecules across the BBB at both trace and

therapeutically relevant doses after a single systemic injection in mice. Distribution of antibody changed from vascular to neuronal 24 hours after injection, indicating that a significant amount of antibody had transcytosed through brain endothelial cells to reach the parenchyma. The magnitude of antibody uptake into and distribution in the CNS was inversely related to its binding affinity to TfR for the anti-TfR variants studied. Proof of BBB transport was achieved using a bispecific antibody that binds both TfR and the amyloid precursor protein (APP) cleavage enzyme, β-secretase (BACE1). A single systemic dose of the bispecific anti-TfR/BACE1 antibody engineered using the methodology of the ~~invention~~disclosure not only resulted in significant antibody uptake in brain, but also dramatically reduced levels of brain $A\beta_{1-40}$ compared to monospecific anti-BACE1 alone, suggesting that BBB penetrance affects the potency of anti-BACE1. Similarly, a bispecific antibody that binds both TfR and amyloid beta (i.e., a portion of APP that results from BACE1 cleavage of APP, which is one of the main constituents of amyloid plaques) was shown to be readily taken up into the brain using the methodology of the ~~invention~~disclosure. The data and experiments described herein highlight several causative mechanisms behind increasing uptake of an antibody into the CNS using a lower-affinity antibody approach. First, high affinity anti-BBB receptor (BBB-R) antibodies (e.g., anti-TfR^A) limit brain uptake by quickly saturating the BBB-R in the brain vasculature, thus reducing the total amount of antibody taken up into the brain and also restricting its distribution to the vasculature. Strikingly, lowering affinity for the BBB-R improves brain uptake and distribution, with a robust shift observed in localization from the vasculature to neurons and associated neuropil distributed within the CNS. Second, the lower affinity of the antibody for the BBB-R is proposed to impair the ability of the antibody to return to the vascular side of the BBB via the BBB-R from the CNS side of the membrane because the overall affinity of the antibody for the BBB-R is low and the local concentration of the antibody on the CNS side of the BBB is non-saturating due to the rapid dispersal of the antibody into the CNS compartment. Third, in vivo, and as observed for the TfR system, antibodies with less affinity for the BBB-R are not cleared from the system as efficiently as those with greater affinity for the BBB-R, and thus remain at higher circulating concentrations than their higher-affinity counterparts. This is advantageous because the circulating antibody levels of the lower-affinity antibody are sustained at therapeutic levels for a longer period of time than the higher-affinity antibody, which consequently improves uptake of antibody in brain for a longer period of time. Furthermore, this improvement in both plasma and brain exposure may reduce the frequency of dosing in the clinic, which would have potential benefit not only for patient compliance and convenience but also in lessening any potential side effects or off-target effects of the antibody

and/or of a therapeutic compound coupled thereto. 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 ~~invention~~disclosure provides methods of engineering BBB-penetrant therapeutics that greatly improve transport across the BBB and CNS distribution of the therapeutic.

Disclosed is~~Accordingly, in a first embodiment, the invention provides~~ a method of transporting a compound across the blood-brain barrier comprising exposing an antibody which binds with low affinity to a blood-brain barrier receptor (BBB-R) coupled to a compound to the blood-brain barrier such that the antibody transports the compound coupled thereto across the blood-brain barrier.

In one aspect, there is provided a method of transporting a compound across the blood-brain barrier comprising exposing an antibody which binds with low affinity to the transferrin receptor (TfR) coupled to a compound to the blood-brain barrier such that the antibody transports the compound coupled thereto across the blood-brain barrier.

In one ~~aspect~~embodiment, the compound is a neurological disorder drug. In another ~~aspect~~embodiment, the compound is an imaging agent. In another ~~aspect~~embodiment, the compound is labeled. In another ~~aspect~~embodiment, the antibody is labeled. In another ~~aspect~~embodiment, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such ~~aspect~~embodiment, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In another ~~aspect~~embodiment, the BBB is in a mammal. In another such ~~aspect~~embodiment, the mammal is a human. In another such ~~aspect~~embodiment, the mammal has a neurological disorder. In another such ~~aspect~~embodiment, the neurological disorder is selected from the group consisting of Alzheimer's disease (AD), stroke, dementia, muscular dystrophy (MD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), cystic fibrosis, Angelman's syndrome, Liddle syndrome, Parkinson's disease, Pick's disease, Paget's disease, cancer, and traumatic brain injury. In another ~~aspect~~embodiment, the BBB is in a human.

In another ~~aspect~~embodiment, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such ~~aspect~~embodiment, the IC50 is from about 5 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 50 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 100 nM to about 100μM. In another ~~aspect~~embodiment, the antibody has an affinity for the BBB-R from about 5 nM to about 10 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 30 nM to about 1 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 50 nM to about 1 μM. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1

antibody. In another such aspectembodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such aspectembodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In one aspectembodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another aspectembodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another aspectembodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another aspectembodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such aspectembodiment, the BBB-R is a human BBB-R. In one such aspectembodiment, the BBB-R is TfR. In another such aspectembodiment, the BBB-R is TfR and the antibody does not inhibit TfR activity. In another such aspectembodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin. In another aspectembodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such aspectembodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds.

In another aspectembodiment, the compound is covalently coupled to the antibody. In one such aspectembodiment, the compound is joined to the antibody by a linker. In one such aspectembodiment, the linker is cleavable. In another such aspectembodiment, the linker is not cleavable. In another such aspectembodiment, the compound is directly linked to the antibody. In one such aspectembodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such aspectembodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such aspectembodiment, 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 E4 (ApoE4), 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. In another such aspectembodiment, the multispecific antibody binds both TfR and BACE1. In another such aspectembodiment, the multispecific antibody binds both TfR and Abeta. In another such aspectembodiment, the multispecific antibody is labeled. In another aspectembodiment, the compound is

reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

Also disclosed is~~In another embodiment, the invention provides~~ a method of increasing exposure of the CNS to a compound, wherein the compound is coupled to an antibody which binds with low affinity to a BBB-R, thereby increasing the exposure of the CNS to the compound. In one ~~aspect~~embodiment, the compound is a neurological disorder drug. In another ~~aspect~~embodiment, the compound is an imaging agent. In another ~~aspect~~embodiment, the compound is labeled. In another ~~aspect~~embodiment, the antibody is labeled. In another ~~aspect~~embodiment, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such ~~aspect~~embodiment, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In another ~~aspect~~embodiment, the antibody-coupled compound is administered to a mammal. In another such ~~aspect~~embodiment, the mammal is a human. In another such ~~aspect~~embodiment, the mammal has a neurological disorder. In another such ~~aspect~~embodiment, the neurological disorder is selected from the group consisting of Alzheimer's disease (AD), stroke, dementia, muscular dystrophy (MD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), cystic fibrosis, Angelman's syndrome, Liddle syndrome, Parkinson's disease, Pick's disease, Paget's disease, cancer, and traumatic brain injury.

In another ~~aspect~~embodiment, the increase in CNS exposure to the compound is measured relative to the CNS exposure of a compound coupled with a typical antibody not having lowered affinity for the BBB-R. In another ~~aspect~~embodiment, the increase in CNS exposure to the compound is measured as a ratio of the amount of the compound found in the CNS relative to the amount found in the serum after administration. In another such ~~aspect~~embodiment, the increase in CNS exposure results in a ratio of greater than 0.1%. In another ~~aspect~~embodiment, the increase in CNS exposure to the compound is measured relative to the CNS exposure of a compound in the absence of a coupled antibody. In another ~~aspect~~embodiment, the increase in CNS exposure to the compound is measured by imaging. In another ~~aspect~~embodiment, the increase in CNS exposure to the compound is measured by an indirect readout such as a modification of one or more physiological symptoms.

In another ~~aspect~~embodiment, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such ~~aspect~~embodiment, the IC50 is from about 5 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 50 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 100 nM to about 100μM. In another ~~aspect~~embodiment, the antibody has an affinity for the BBB-R from about 5 nM to about 10 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 30 nM to about 1 μM. In another

such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 50 nM to about 1 μM. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In one ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another ~~aspect~~embodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such ~~aspect~~embodiment, the BBB-R is a human BBB-R. In one such ~~aspect~~embodiment, the BBB-R is TfR. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit TfR activity. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin. In another ~~aspect~~embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such ~~aspect~~embodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds.

In another ~~aspect~~embodiment, the compound is covalently coupled to the antibody. In one such ~~aspect~~embodiment, the compound is joined to the antibody by a linker. In one such ~~aspect~~embodiment, the linker is cleavable. In another such ~~aspect~~embodiment, the linker is not cleavable. In another such ~~aspect~~embodiment, the compound is directly linked to the antibody. In one such ~~aspect~~embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such ~~aspect~~embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such ~~aspect~~embodiment, 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 E4 (ApoE4), 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. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and BACE1. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and Abeta. In another such ~~aspect~~embodiment, the multispecific antibody is labeled. In another ~~aspect~~embodiment, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

In another embodiment, the ~~invention~~disclosure provides a method of decreasing clearance of a compound administered to a subject, wherein the compound is coupled to an antibody which binds with low affinity to a BBB-R, such that the clearance of the compound is decreased. In one ~~aspect~~embodiment, the compound is a neurological disorder drug. In another ~~aspect~~embodiment, the compound is an imaging agent. In another ~~aspect~~embodiment, the compound is labeled. In another ~~aspect~~embodiment, the antibody is labeled. In another ~~aspect~~embodiment, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such ~~aspect~~embodiment, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In another ~~aspect~~embodiment, the subject is a mammal. In another such ~~aspect~~embodiment, the mammal is a human. In another such ~~aspect~~embodiment, the mammal has a neurological disorder. In another such ~~aspect~~embodiment, the neurological disorder is selected from the group consisting of Alzheimer's disease (AD), stroke, dementia, muscular dystrophy (MD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), cystic fibrosis, Angelman's syndrome, Liddle syndrome, Parkinson's disease, Pick's disease, Paget's disease, cancer, and traumatic brain injury.

In another ~~aspect~~embodiment, the decrease in clearance of the compound is measured relative to the clearance of a compound coupled with a typical antibody not having lowered affinity for the BBB-R. In another ~~aspect~~embodiment, the decrease in clearance of the compound is measured relative to the clearance of the compound in the absence of a coupled antibody.

In another ~~aspect~~embodiment, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such ~~aspect~~embodiment, the IC50 is from about 5 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 50 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 100 nM to about 100μM. In another ~~aspect~~embodiment, the antibody has an affinity for the BBB-R from about 5 nM to about 10 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 30 nM to about 1 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 50 nM to about 1 μM. In another such ~~aspect~~embodiment, the compound-coupled antibody

specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In one ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another ~~aspect~~embodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such ~~aspect~~embodiment, the BBB-R is a human BBB-R. In one such ~~aspect~~embodiment, the BBB-R is TfR. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit TfR activity. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin. In another ~~aspect~~embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such ~~aspect~~embodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds.

In another ~~aspect~~embodiment, the compound is covalently coupled to the antibody. In one such ~~aspect~~embodiment, the compound is joined to the antibody by a linker. In one such ~~aspect~~embodiment, the linker is cleavable. In another such ~~aspect~~embodiment, the linker is not cleavable. In another such ~~aspect~~embodiment, the compound is directly linked to the antibody. In one such ~~aspect~~embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such ~~aspect~~embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such ~~aspect~~embodiment, 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 E4 (ApoE4), 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. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and BACE1. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and Abeta. In another such ~~aspect~~embodiment, the multispecific antibody is labeled. In another ~~aspect~~embodiment, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

A method of increasing retention in the CNS of a compound administered to a subject, wherein the compound is coupled to an antibody which binds with low affinity to a BBB-R, such that the retention in the CNS of the compound is increased. In one ~~aspect~~embodiment, the compound is a neurological disorder drug. In another ~~aspect~~embodiment, the compound is an imaging agent. In another ~~aspect~~embodiment, the compound is labeled. In another ~~aspect~~embodiment, the antibody is labeled. In another ~~aspect~~embodiment, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such ~~aspect~~embodiment, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In another ~~aspect~~embodiment, the compound is administered to a mammal. In another such ~~aspect~~embodiment, the mammal is a human. In another such ~~aspect~~embodiment, the mammal has a neurological disorder. In another such ~~aspect~~embodiment, the neurological disorder is selected from the group consisting of Alzheimer's disease (AD), stroke, dementia, muscular dystrophy (MD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), cystic fibrosis, Angelman's syndrome, Liddle syndrome, Parkinson's disease, Pick's disease, Paget's disease, cancer, and traumatic brain injury.

In another ~~aspect~~embodiment, the increase in CNS retention of the compound is measured relative to the CNS retention of a compound coupled with a typical antibody not having lowered affinity for the BBB-R. In another ~~aspect~~embodiment, the increase in CNS retention of the compound is measured as a ratio of the amount of the compound found in the CNS relative to the amount found in the serum at one or more time points after administration. In another such ~~aspect~~embodiment, the increase in CNS retention results in a ratio of greater than 0.1% at one or more time points after administration. In another ~~aspect~~embodiment, the increase in CNS retention of the compound is measured relative to the CNS retention of a compound in the absence of a coupled antibody. In another ~~aspect~~embodiment, the increase in CNS retention of the compound is measured by imaging. In another ~~aspect~~embodiment, the increase in CNS retention of the compound is measured by an indirect readout such as a modification of one or more physiological symptoms.

In another ~~aspect~~embodiment, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such ~~aspect~~embodiment, the IC50 is from about 5 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 50 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50

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In another ~~aspect~~embodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such ~~aspect~~embodiment, the BBB-R is a human BBB-R. In one such ~~aspect~~embodiment, the BBB-R is TfR. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit TfR activity. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin. In another ~~aspect~~embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such ~~aspect~~embodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds.

In another ~~aspect~~embodiment, the compound is covalently coupled to the antibody. In one such ~~aspect~~embodiment, the compound is joined to the antibody by a linker. In one such ~~aspect~~embodiment, the linker is cleavable. In another such ~~aspect~~embodiment, the linker is not cleavable. In another such ~~aspect~~embodiment, the compound is directly linked to the antibody. In one such ~~aspect~~embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such ~~aspect~~embodiment, the multispecific antibody comprises a first antigen binding site which

binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such ~~aspect~~embodiment, 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 E4 (ApoE4), 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. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and BACE1. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and Abeta. In another such ~~aspect~~embodiment, the multispecific antibody is labeled. In another ~~aspect~~embodiment, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

In another embodiment, the ~~invention~~disclosure provides a method of optimizing the pharmcokinetics and/or pharmacodynamics of a compound to be efficacious in the CNS of a subject, wherein the compound is coupled to an antibody which binds with low affinity to a BBB-R, and the antibody is selected such that its affinity for the BBB-R after coupling to the compound results in an amount of transport of the antibody conjugated to the compound across the BBB that optimizes the pharmacokinetics and/or pharmacodynamics of the compound in the CNS. In one ~~aspect~~embodiment, the compound is a neurological disorder drug. In another ~~aspect~~embodiment, the compound is an imaging agent. In another ~~aspect~~embodiment, the compound is labeled. In another ~~aspect~~embodiment, the antibody is labeled. In another ~~aspect~~embodiment, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such ~~aspect~~embodiment, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In another ~~aspect~~embodiment, the BBB is in a mammal. In another such ~~aspect~~embodiment, the mammal is a human. In another such ~~aspect~~embodiment, the mammal has a neurological disorder. In another such ~~aspect~~embodiment, the neurological disorder is selected from the group consisting of Alzheimer's disease (AD), stroke, dementia, muscular dystrophy (MD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), cystic fibrosis, Angelman's syndrome, Liddle syndrome, Parkinson's disease, Pick's disease, Paget's disease, cancer, and traumatic brain injury. In another ~~aspect~~embodiment, the BBB is in a human.

In one ~~aspect~~embodiment, the optimizing may include the generation of a series of antibody-compound complexes in which each antibody has a different affinity for the BBB-R, and assessing the pharmacokinetics and/or pharmacodynamics of each in the CNS. In another ~~aspect~~embodiment, optimizing may be relative to a known standard, such as, but not limited to, the

pharmacokinetics and/or pharmacodynamics of the compound when directly introduced into the CNS or when introduced to the subject in the absence of a coupled anti-BBB-R antibody.

In another ~~aspect~~embodiment, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such ~~aspect~~embodiment, the IC50 is from about 5 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 50 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 100 nM to about 100μM. In another ~~aspect~~embodiment, the antibody has an affinity for the BBB-R from about 5 nM to about 10 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 30 nM to about 1 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 50 nM to about 1 μM. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In one ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another ~~aspect~~embodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such ~~aspect~~embodiment, the BBB-R is a human BBB-R. In one such ~~aspect~~embodiment, the BBB-R is TfR. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit TfR activity. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin. In another ~~aspect~~embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such ~~aspect~~embodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds.

In another ~~aspect~~embodiment, the compound is covalently coupled to the antibody. In one such ~~aspect~~embodiment, the compound is joined to the antibody by a linker. In one such ~~aspect~~embodiment, the linker is cleavable. In another such ~~aspect~~embodiment, the linker is not cleavable. In another such ~~aspect~~embodiment, the compound is directly linked to the antibody. In one such ~~aspect~~embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such ~~aspect~~embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such ~~aspect~~embodiment, 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 E4 (ApoE4), 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. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and BACE1. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and Abeta. In another such ~~aspect~~embodiment, the multispecific antibody is labeled. In another ~~aspect~~embodiment, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

Also disclosed is~~In another embodiment the invention provides~~ a method of treating a neurological disorder in a mammal comprising treating the mammal with an antibody that binds a BBB-R and is coupled to a compound, wherein the antibody has been selected to have a low affinity for the BBB-R and thereby improves CNS uptake of the antibody and coupled compound. In one ~~aspect~~embodiment, the compound is a neurological disorder drug. In another ~~aspect~~embodiment, the compound is an imaging agent. In another ~~aspect~~embodiment, the compound is labeled. In another ~~aspect~~embodiment, the antibody is labeled. In another ~~aspect~~embodiment, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such ~~aspect~~embodiment, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In one ~~aspect~~embodiment, the mammal is a human. In another such ~~aspect~~embodiment, the mammal has a neurological disorder. In another such ~~aspect~~embodiment, the neurological disorder is selected from the group consisting of Alzheimer's disease (AD), stroke, dementia, muscular dystrophy (MD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), cystic fibrosis, Angelman's syndrome, Liddle syndrome, Parkinson's disease, Pick's disease, Paget's disease, cancer, and traumatic brain injury.

In one ~~aspect~~embodiment, the treating results in lessening or elimination of disorder symptoms. In another ~~aspect~~embodiment, the treating results in amelioration of the neurological disorder.

In another ~~aspect~~embodiment, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such ~~aspect~~embodiment, the IC50 is from about 5 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 50 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 100 nM to about 100μM. In another ~~aspect~~embodiment, the antibody has an affinity for the BBB-R from about 5 nM to about 10 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 30 nM to about 1 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 50 nM to about 1 μM. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In one ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another ~~aspect~~embodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such ~~aspect~~embodiment, the BBB-R is a human BBB-R. In one such ~~aspect~~embodiment, the BBB-R is TfR. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit TfR activity. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin. In another ~~aspect~~embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such ~~aspect~~embodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds.

In another ~~aspect~~embodiment, the compound is covalently coupled to the antibody. In one such ~~aspect~~embodiment, the compound is joined to the antibody by a linker. In one such ~~aspect~~embodiment, the linker is cleavable. In another such ~~aspect~~embodiment, the linker is not cleavable. In another such ~~aspect~~embodiment, the

compound is directly linked to the antibody. In one such ~~aspect~~embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such ~~aspect~~embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such ~~aspect~~embodiment, 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 E4 (ApoE4), 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. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and BACE1. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and Abeta. In another such ~~aspect~~embodiment, the multispecific antibody is labeled. In another ~~aspect~~embodiment, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

In another embodiment, the ~~invention~~disclosure provides a method of making an antibody useful for transporting a compound across the BBB comprising selecting an antibody specific for a blood-brain barrier receptor (BBB-R) because it has a desirably low affinity for the BBB-R.

In one ~~aspect~~embodiment, the antibody is selected from a panel of antibodies based upon the affinity of the selected antibody. In another ~~aspect~~embodiment, the antibody is engineered to have the affinity. In one such ~~aspect~~embodiment, the antibody is generated using any art-known protein engineering methodology including, but not limited to, phage display, yeast display, random mutagenesis, and site-directed mutagenesis.

In one ~~aspect~~embodiment, the compound is a neurological disorder drug. In another ~~aspect~~embodiment, the compound is an imaging agent. In another ~~aspect~~embodiment, the compound is labeled. In another ~~aspect~~embodiment, the antibody is labeled. In another ~~aspect~~embodiment, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such ~~aspect~~embodiment, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In another ~~aspect~~embodiment, the BBB is in a mammal. In another such ~~aspect~~embodiment, the mammal is a human. In another such ~~aspect~~embodiment, the mammal has a neurological disorder. In another such ~~aspect~~embodiment, the neurological disorder is selected from the group consisting of Alzheimer's disease (AD), stroke, dementia, muscular dystrophy (MD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), cystic fibrosis, Angelman's syndrome, Liddle syndrome, Parkinson's disease, Pick's disease, Paget's disease, cancer, and traumatic brain injury. In another ~~aspect~~embodiment, the BBB is in a human.

In another ~~aspect~~embodiment, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such ~~aspect~~embodiment, the IC50 is from about 5 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 50 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 100 nM to about 100μM. In another ~~aspect~~embodiment, the antibody has an affinity for the BBB-R from about 5 nM to about 10 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 30 nM to about 1 μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 50 nM to about 1 μM. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In one ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another ~~aspect~~embodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such ~~aspect~~embodiment, the BBB-R is a human BBB-R. In one such ~~aspect~~embodiment, the BBB-R is TfR. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit TfR activity. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin. In another ~~aspect~~embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such ~~aspect~~embodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds.

In another ~~aspect~~embodiment, the compound is covalently coupled to the antibody. In one such ~~aspect~~embodiment, the compound is joined to the antibody by a linker. In one such ~~aspect~~embodiment, the linker is cleavable. In another such ~~aspect~~embodiment, the linker is not cleavable. In another such ~~aspect~~embodiment, the

compound is directly linked to the antibody. In one such ~~aspect~~embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such ~~aspect~~embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such ~~aspect~~embodiment, 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 E4 (ApoE4), 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. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and BACE1. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and Abeta. In another such ~~aspect~~embodiment, the multispecific antibody is labeled. In another ~~aspect~~embodiment, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

In another embodiment, the ~~invention~~disclosure provides an antibody which binds to a blood-brain barrier receptor (BBB-R) with low affinity. In one ~~aspect~~embodiment, the affinity of the antibody for the BBB-R is from about 5 nM to about 10 μM. In another ~~aspect~~embodiment, the affinity of the antibody for the BBB-R is from about 20 nM to about 1 μM. In another ~~aspect~~embodiment, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such ~~aspect~~embodiment, the IC50 is from about 5 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 50 nM to about 100μM. In another such ~~aspect~~embodiment, the IC50 is from about 100 nM to about 100μM. In another such ~~aspect~~embodiment, the antibody, when coupled to a compound, has an affinity for the BBB-R from about 50 nM to about 1 μM. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an affinity for TfR between those affinities observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In another such ~~aspect~~embodiment, the compound-coupled antibody specifically binds to TfR and has an IC50 for TfR between those IC50s observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody. In one ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-

BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another ~~aspect~~embodiment, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another ~~aspect~~embodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 8 (LRP8), low density lipoprotein receptor-related protein 1 (LRP1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In one such ~~aspect~~embodiment, the BBB-R is TfR. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit TfR activity. In another such ~~aspect~~embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin. In another such ~~aspect~~embodiment, the BBB-R is a human BBB-R.

In another ~~aspect~~embodiment, the antibody is coupled to a compound. In one ~~aspect~~embodiment, the compound is a neurological disorder drug. In another ~~aspect~~embodiment, the compound is an imaging agent. In another ~~aspect~~embodiment, the compound is labeled. In another ~~aspect~~embodiment, the antibody is labeled. In another ~~aspect~~embodiment, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such ~~aspect~~embodiment, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin.

In another ~~aspect~~embodiment, the compound is covalently coupled to the antibody. In one such ~~aspect~~embodiment, the compound is joined to the antibody by a linker. In one such ~~aspect~~embodiment, the linker is cleavable. In another such ~~aspect~~embodiment, the linker is not cleavable. In another such ~~aspect~~embodiment, the compound is directly linked to the antibody. In one such ~~aspect~~embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such ~~aspect~~embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such ~~aspect~~embodiment, 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 E4 (ApoE4), 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. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and BACE1. In another such ~~aspect~~embodiment, the multispecific antibody binds both TfR and Abeta. In another such ~~aspect~~embodiment, the multispecific antibody is labeled. In another ~~aspect~~embodiment, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

In another ~~aspect~~embodiment, the antibody is an antibody fragment with an antigen-binding region that binds the BBB-R, including, but not limited to, Fab, Fab', $F(ab')_2$, and Fv. In another ~~aspect~~embodiment, the antibody is a full-length antibody.

Also disclosed is~~In another embodiment, the invention provides~~ the use of an antibody that binds with low affinity to a BBB-R for the manufacture of a medicament for treating a neurological disorder. In one aspect, there is provided the use of an antibody in the manufacture of a medicament for treatment of a neurological disorder in a mammal wherein the antibody is one that binds TfR and is coupled to a compound, wherein the antibody has been selected to have a low affinity for TfR and thereby improves CNS uptake of the antibody and coupled compound. Any of the foregoing described low-affinity anti-BBB-R antibodies or any of the low-affinity anti-BBB-R antibodies described elsewhere herein may be used in the method.

Further disclosed is~~In another embodiment, the invention provides~~ an antibody that binds with low affinity to a BBB-R for use in treating a neurological disorder. In one aspect, there is provided an agent for use in the treatment of a neurological disorder in a mammal wherein the agent comprises an antibody that binds TfR and is coupled to a compound, wherein the antibody has been selected to have a low affinity for TfR and thereby improves CNS uptake of the antibody and coupled compound. Any of the foregoing described low-affinity anti-BBB-R antibodies or any of the low-affinity anti-BBB-R antibodies described elsewhere herein may be used in the method. Accordingly, in a first ~~aspect~~embodiment, the ~~invention~~disclosure provides an antibody which binds to a blood-brain barrier receptor (BBB-R), wherein the affinity of the antibody for the BBB-R is from about 5 nM to about 10 μM (e.g. from about 20 nM to about 1μM). Optionally, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor, insulin-like growth factor receptor (IGF receptor), low density lipoprotein receptor-related protein 1 (LRP1), low density lipoprotein receptor-related protein 8 (LRP8), and heparin-binding epidermal growth factor-like growth factor (HB-EGF). Optionally, the antibody is coupled with a therapeutic compound, such as a neurological disorder drug. In one embodiment, the antibody is a multispecific antibody which comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen, for instance where the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), amyloid beta (Abeta), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein A3 (ApoE3), apolipoprotein E4 (ApoE4), 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. The antibody (e.g. multispecific antibody) includes antibody fragments and full-length antibodies.

In another embodiment, the ~~invention~~disclosure provides a method of transporting a therapeutic compound, such as a neurological disorder drug, across the blood-brain barrier comprising exposing the anti-BBB-R antibody coupled with a neurological disorder drug to the blood-brain barrier such that the antibody transports the neurological disorder drug coupled thereto across the blood-brain barrier. The blood-brain barrier in this method may be in a mammal, e.g. one with a neurological disorder, examples of which include: Alzheimer's disease (AD)

(including, but not limited to, mild cognitive impairment and prodromal AD), stroke, dementia, muscular dystrophy (MD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), cystic fibrosis, Angelman's syndrome, Liddle syndrome, Parkinson's disease, Pick's disease, Paget's disease, cancer (e.g. cancer affecting the CNS or brain), and traumatic brain injury.

The ~~invention~~disclosure additionally concerns a method of making an antibody useful for transporting a therapeutic compound such as a neurological disorder drug across the blood-brain barrier comprising selecting an antibody against a blood-brain barrier receptor (BBB-R) because it has an affinity for the BBB-R which is from about 5 nM to about 10 μM. In one embodiment the antibody is selected from a panel of antibodies because it has the desired affinity. Alternatively, or additionally, the antibody is engineered to have the desired affinity. The method optionally further comprises coupling the antibody with a therapeutic compound such as a neurological disorder drug. For example, the method can comprise making a multispecific antibody which comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen.

The ~~invention~~disclosure additionally provides a method of treating a neurological disorder in a mammal comprising treating the mammal with a multispecific antibody that binds both a blood-brain barrier receptor (BBB-R) and a brain antigen, wherein the anti-BBB-R antibody has been selected to have a low affinity for the BBB-R and thereby improves brain uptake of the anti-brain antigen antibody. Optionally, the multispecific antibody binds both transferrin receptor (TfR) and BACE1 or Abeta.

It will be understood that any of the foregoing methods and compositions of the ~~invention~~disclosure may be combined with one another and/or with the further ~~aspects~~embodiments of the ~~invention~~disclosure described in the specification herein.

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-E depict significant brain vascular uptake of systemically administered anti-TfR antibody. Fig. 1A shows brain uptake after IV administration of trace doses (approximately 50 μg/kg) of [^{131}I]anti-TfR^{A} and [^{125}I]control IgG in mice and was quantified as a mean percentage of injected dose per gram of brain at 5, 30 min., 1, 4, 24, 48, and 72 hours after IV injection (n=6). Uptake of [^{131}I]anti-TfR^{A} was decreased by injection with 4 mg/kg unlabeled anti-TfR^{A} (cold). Fig. 1B shows quantification of mean antibody uptake in brain 1 and 24 hours after a 20mg/kg IV injection of control IgG or anti-TfR^{A} (***p=0.0002, n=10). Fig. 1C shows ratio of mean percent brain to serum concentrations (**p=0.003, n=10). Figs. 1D and 1E depict immunohistochemical staining of brain sections following IV-injection with