receptors (ie, transferrin receptor, insulin receptor, low density lipoprotein receptor-related protein 8, glucose transporter 1 (Glut1) and the like) (see, e.g., WO9502421). For example, the anti-BBB receptor antibody can be made multispecific to target one or more desired antigens in the CNS, or one or more heterologous molecules can be coupled to the anti-BBB receptor antibody; in either case, the anti-BBB receptor antibody can assist in delivering a therapeutic molecule into the CNS across the BBB.

However, targeting a BBB receptor with a traditional specific high-affinity antibody generally resulted in limited increase in BBB transport. It was later found by Applicants that the magnitude of antibody uptake into and distribution in the CNS is inversely related to its binding affinity for the BBB receptor amongst the anti-BBB antibodies studied. For example, a low-affinity antibody to transferrin receptor (TfR) dosed at therapeutic dose levels greatly improves BBB transport and CNS retention of the anti-TfR antibody relative to a higher-affinity anti-TfR antibody, and makes it possible to more readily attain therapeutic concentrations in the CNS (Atwal et al., *Sci. Transl. Med.* **3**, 84ra43 (2011)). Proof of such 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 $A\beta_{1\text{-}40}$ compared to monospecific anti-BACE1 alone, suggesting that BBB penetrance affects the potency of anti-BACE1. (Atwal et al., *Sci. Transl. Med.* **3**, 84ra43 (2011); Yu et al., *Sci. Transl. Med.* **3**, 84ra44 (2011)).

Those data and experiments highlighted 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.

The low-affinity BBB-R antibodies described in the above-referenced work were selected /engineered to avoid interference with the natural binding between transferrin and the TfR, and thus to avoid potential iron transport-related side effects. Nonetheless, upon administration of certain of these antibodies in mice, some marked side effects were observed. The mice displayed a primary response of robust depletion of reticulocyte populations accompanied by rapid onset acute clinical symptoms, as described in the Examples. Further in vitro studies using a human erythroblast cell line and primary bone marrow cells treated with anti-human TfR antibodies demonstrated that a robust depletion of TfR-positive erythroid cells is also observable in human cellular systems (see, e.g., Example 7). Though the mice recovered from both the acute clinical symptoms and the decreased reticulocyte levels in due course, avoiding or otherwise mitigating this impact on reticulocytes is clearly desirable for an anti-TfR antibody to be able to be used safely as a therapeutic molecule.

Accordingly, the disclosure~~invention~~ provides compositions and methods that greatly reduce or eliminate the unwanted reduction in the reticulocyte population upon anti-TfR administration while still enabling the enhanced BBB transport, increased CNS distribution and CNS retention provided by low-affinity anti-TfR antibodies administered at therapeutic concentrations. The results described herein show that the primary response to anti-TfR administration (robust reticulocyte depletion and acute clinical signs) is driven in large part by the antibody-dependent cell-mediated cytotoxicity (ADCC) activity of the antibody, while the residual reticulocyte depletion effect is mediated by the complement pathway. Several general approaches to mitigate the observed effect of anti-TfR antibodies on both the primary and residual reticulocyte depletion are provided herein, and may be used singly or in combination.

In one approach, the effector function of the anti-BBB-R antibody is reduced or eliminated in order to reduce or eliminate ADCC activity. In another approach, the affinity of the anti-BBB-R antibody for the BBB-R is further lessened such that interactions of the

maintained for efficacy, while still below the threshold of the cell-depleting side effect. Another method, which may be combined with dosing modifications, is to select or engineer an anti-TfR antibody that has pH-sensitive binding to TfR such that it binds to cell surface TfR in the plasma at pH 7.4 with desirably low affinity as described herein, but upon internalization into an endosomal compartment, such binding to TfR is rapidly and significantly reduced at the relatively lower pH of that compartment (pH 5.5-6.0). Such dissociation may protect the antibody from antigen-mediated clearance, or increase the amount of antibody that is either delivered to the CNS or recycled back across the BBB – in either case, the effective concentration of the antibody is increased relative to an anti-TfR antibody that does not comprise such pH sensitivity, without increasing the administered dose of the antibody.

Protecting, stabilizing and/or replenishing reticulocyte populations may be accomplished using pharmaceutical or physical methods. In addition to the anti-BBB-R antibody, at least one further therapeutic agent may be coadministered (simultaneously or sequentially) that mitigates negative side effects of the antibody on reticulocyte populations. Examples of such therapeutic agents include, but are not limited to, erythropoietin (EPO), iron supplements, vitamin C, folic acid, and vitamin B12. Physical replacement of red blood cells (ie, reticulocytes) is also possible by, for example, transfusion with similar cells, which may be from another individual of similar blood type or may have been previously extracted from the subject to whom the anti-BBB-R antibody is administered.

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 the BBB-R 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-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-BBB-R antibody; and (iv) 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-TfR antibody of the disclosure~~invention~~ 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~~invention~~ provides methods of engineering BBB-penetrant therapeutics that greatly improve transport across the BBB and CNS distribution of the therapeutic without depletion of reticulocytes.

Accordingly, in ~~a first embodiment, the invention~~one aspect there is provided~~s~~ a method of transporting a compound across the blood-brain barrier in a subject 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, wherein reduction of red blood cell levels in the subject upon antibody administration to the subject is decreased or eliminated. In one ~~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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodiment~~aspect~~, the BBB-R is a human BBB-R. In one such embodiment~~aspect~~, the BBB-R is TfR. In another such embodiment~~aspect~~, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodiment~~aspect~~, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodiment~~aspect~~, the red blood cells are immature red blood cells. In another such embodiment~~aspect~~, the immature red blood cells are reticulocytes. In another embodiment~~aspect~~, reduction of reticulocyte levels is accompanied by acute clinical symptoms. In another embodiment~~aspect~~, the method further comprises the step of monitoring the subject for depletion of red blood cells.

In another embodiment~~aspect~~, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodiment~~aspect~~, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodiment~~aspect~~, the effector function of the antibody Fc region is modified. In one such embodiment~~aspect~~, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In

another such embodimentaspect, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodimentaspect, the antibody is produced in a non-mammalian cell production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the Fc region and/or the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, complement-triggering 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 that engages the complement pathway. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody. In another such embodimentaspect, the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such

embodimentaspect, the modification is a point mutation of the CH1 region to impair binding to C3. In one such embodimentaspect, the point mutation is at position 132 (see, e.g., Vidarte et al., (2001) J. Biol. Chem. 276(41): 38217-38223).

In another embodimentaspect, the dose amount and/or frequency of administration of the antibody is modulated to reduce the concentration of the antibody to which the red blood cells are exposed. In another embodimentaspect, the antibody is modified to comprise pH-sensitive binding to the BBB-R.

In another embodimentaspect, a further compound is administered in addition to the antibody and the coupled compound. In one such embodimentaspect, the further compound is responsible for or contributes to the lack of reduction of reticulocyte levels. In another such embodimentaspect, the further compound inhibits or prevents the activation or activity of the complement pathway (see, e.g., Mollnes and Kirschfink (2006) Molec. Immunol. 43:107-121). In another such embodimentaspect, the further compound protects reticulocytes from antibody-related depletion. In another such embodimentaspect, the further compound supports the growth, development, or reestablishment of reticulocytes. In another embodimentaspect, the further compound is selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid and vitamin B12. In another embodimentaspect, the further compound is red blood cells or reticulocytes from the same subject. In another embodimentaspect, the further compound is red blood cells or reticulocytes from another subject.

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

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

BBB-R from about 5 nM to about 50 μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such embodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In

another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA. In another embodimentaspect, the compound-coupled antibody is administered at a therapeutic dose. In one such aspect, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodimentaspect, the compound-coupled antibody is administered at a dose and dose frequency that minimizes red blood cell interaction with the compound-coupled antibody while still facilitating compound delivery across the BBB into the CNS at therapeutic levels.

In another embodimentaspect, the compound is covalently coupled to the antibody. In one such embodimentaspect, the compound is joined to the antibody by a linker. In one such embodimentaspect, the linker is cleavable. In another such embodimentaspect, the linker is not cleavable. In another such embodimentaspect, the compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

In ~~another embodiment, the invention~~one aspect, there is provideds a method of increasing exposure of the CNS of a subject 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, and wherein reduction of red blood cell levels in the subject upon compound-coupled antibody administration to the subject is decreased or eliminated. In one embodimentaspect, 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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodimentaspect, the BBB-R is a human BBB-R. In one such embodimentaspect, the BBB-R is TfR. In another such embodimentaspect, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodimentaspect, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodimentaspect, the red blood cells are immature red blood cells. In another such embodimentaspect, the immature red blood cells are reticulocytes. In another embodimentaspect, reduction of reticulocyte levels is accompanied by acute clinical symptoms. In another embodimentaspect, the method further comprises the step of monitoring the subject for depletion of red blood cells.

In another embodimentaspect, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodimentaspect, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodimentaspect, the effector function of the antibody Fc region is modified. In one such embodimentaspect, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In another such embodimentaspect, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodimentaspect, the antibody is produced in a non-mammalian cell production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the Fc region and/or the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, complement-triggering 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 that engages the complement pathway. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody. In another such embodimentaspect, the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the CH1 region to impair binding to C3. In one such embodimentaspect, the point mutation is at position 132 (see, e.g., Vidarte et al., (2001) J. Biol. Chem. 276(41): 38217-38223).

In another embodimentaspect, the dose amount and/or frequency of administration of the antibody is modulated to reduce the concentration of the antibody to which the red blood cells are exposed. In another embodimentaspect, the antibody is modified to comprise pH-sensitive binding to the BBB-R.

In another embodimentaspect, a further compound is administered in addition to the antibody and the coupled compound. In one such embodimentaspect, the further compound is responsible for or contributes to the lack of reduction of reticulocyte levels. In another such embodimentaspect, the further compound inhibits or prevents the activation or activity of the complement pathway (see, e.g., Mollnes and Kirschfink (2006) Molec. Immunol. 43:107-121). In another such embodimentaspect, the further compound protects reticulocytes from antibody-related depletion. In another such embodimentaspect, the further compound supports the growth, development, or reestablishment of reticulocytes. In another embodimentaspect, the further compound is selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid and vitamin B12. In another embodimentaspect, the further

compound is red blood cells or reticulocytes from the same subject. In another embodimentaspect, the further compound is red blood cells or reticulocytes from another subject.

In another embodimentaspect, the compound is a neurological disorder drug. In another embodimentaspect, the compound is an imaging agent. In another embodimentaspect, the compound is labeled. In another embodimentaspect, the antibody is labeled. In another embodimentaspect, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such embodimentaspect, the antibody specifically binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In another embodimentaspect, the antibody-coupled compound is administered to a mammal. In another such embodimentaspect, the mammal is a human. In another such embodimentaspect, the mammal has a neurological disorder. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the increase in CNS exposure results in a ratio of greater than 0.1%. In another embodimentaspect, 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 embodimentaspect, the increase in CNS exposure to the compound is measured by imaging. In another embodimentaspect, 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 embodimentaspect, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such embodimentaspect, the IC50 is from about 5 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 50 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 100 nM to about 100μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such embodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA.

In another embodimentaspect, the compound-coupled antibody is administered at a therapeutic dose. In one such embodimentaspect, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodimentaspect, the compound-coupled antibody is

administered at a dose and dose frequency that minimizes red blood cell interaction with the compound-coupled antibody while still facilitating compound delivery across the BBB into the CNS at therapeutic levels.

In another embodimentaspect, the compound is covalently coupled to the antibody. In one such embodimentaspect, the compound is joined to the antibody by a linker. In one such embodimentaspect, the linker is cleavable. In another such embodimentaspect, the linker is not cleavable. In another such embodimentaspect, the compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

In ~~another embodiment, the invention~~one aspect, there is provideds 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, and wherein reduction of red blood cell levels in the subject upon compound-coupled antibody administration to the subject is decreased or eliminated. In one embodimentaspect, 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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodimentaspect, the BBB-R is a human BBB-R. In one such embodimentaspect, the BBB-R is TfR. In another such embodimentaspect, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another

such embodimentaspect, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodimentaspect, the red blood cells are immature red blood cells. In another such embodimentaspect, the immature red blood cells are reticulocytes. In another embodimentaspect, reduction of reticulocyte levels is accompanied by acute clinical symptoms. In another embodimentaspect, the method further comprises the step of monitoring the subject for depletion of red blood cells.

In another embodimentaspect, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodimentaspect, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodimentaspect, the effector function of the antibody Fc region is modified. In one such embodimentaspect, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In another such embodimentaspect, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodimentaspect, the antibody is produced in a non-mammalian cell production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the Fc region and/or the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, complement-triggering 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 that engages the complement pathway. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody. In another such embodimentaspect, the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the CH1 region to impair binding to C3. In one such embodimentaspect, the point mutation is at position 132 (see, e.g., Vidarte et al., (2001) J. Biol. Chem. 276(41): 38217-38223).

In another embodimentaspect, the dose amount and/or frequency of administration of the antibody is modulated to reduce the concentration of the antibody to which the red blood cells are exposed. In another embodimentaspect, the antibody is modified to comprise pH-sensitive binding to the BBB-R.

In another embodimentaspect, a further compound is administered in addition to the antibody and the coupled compound. In one such embodimentaspect, the further compound is responsible for or contributes to the lack of reduction of reticulocyte levels. In another such embodimentaspect, the further compound inhibits or prevents the activation or activity of the complement pathway (see, e.g., Mollnes and Kirschfink (2006) Molec. Immunol. 43:107-121). In another such embodimentaspect, the further compound protects reticulocytes from antibody-related depletion. In another such embodimentaspect, the further compound supports the growth, development, or reestablishment of reticulocytes. In another embodimentaspect, the further compound is selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid and vitamin B12. In another embodimentaspect, the further compound is red blood cells or reticulocytes from the same subject. In another embodimentaspect, the further compound is red blood cells or reticulocytes from another subject.

In another embodimentaspect, the compound is a neurological disorder drug. In another embodimentaspect, the compound is an imaging agent. In another embodimentaspect, the compound is labeled. In another embodimentaspect, the antibody is labeled. In another embodimentaspect, the antibody does not impair the binding of the BBB-R to one or more of its native ligands. In another such embodimentaspect, the antibody specifically

binds to TfR in such a manner that it does not inhibit binding of the TfR to transferrin. In another embodimentaspect, the subject is a mammal. In another such embodimentaspect, the mammal is a human. In another such embodimentaspect, the mammal has a neurological disorder. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such embodimentaspect, the IC50 is from about 5 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 50 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 100 nM to about 100μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such embodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA.

In another embodimentaspect, the compound-coupled antibody is administered at a therapeutic dose. In one such embodimentaspect, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodimentaspect, the compound-coupled antibody is administered at a dose and dose frequency that minimizes red blood cell interaction with the compound-coupled antibody while still facilitating compound delivery across the BBB into the CNS at therapeutic levels.

In another embodimentaspect, the compound is covalently coupled to the antibody. In one such embodimentaspect, the compound is joined to the antibody by a linker. In one such embodimentaspect, the linker is cleavable. In another such embodimentaspect, the linker is not cleavable. In another such embodimentaspect, the compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

AIn one aspect, there is provided 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, and wherein reduction of red blood cell levels in the subject upon compound-coupled antibody administration to the subject is decreased or eliminated. In one embodimentaspect, 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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodimentaspect, the BBB-R is a human BBB-R. In one such embodimentaspect, the BBB-R is TfR. In another such embodimentaspect, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodimentaspect, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodimentaspect, the red blood cells are immature red blood cells. In another such embodimentaspect, the immature red blood cells are reticulocytes. In another embodimentaspect, reduction of reticulocyte levels is accompanied by acute clinical symptoms. In another embodimentaspect, the method further comprises the step of monitoring the subject for depletion of red blood cells.

In another embodimentaspect, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodimentaspect, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodimentaspect, the effector function of the antibody Fc region is modified. In one such embodimentaspect, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In another such embodimentaspect, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodimentaspect, the glycosylation of the antibody is

reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodimentaspect, the antibody is produced in a non-mammalian cell production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the Fc region and/or the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, complement-triggering 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 that engages the complement pathway. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody. In another such embodimentaspect, the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the CH1 region to impair binding to C3. In one

such embodimentaspect, the point mutation is at position 132 (see, e.g., Vidarte et al., (2001) J. Biol. Chem. 276(41): 38217-38223).

In another embodimentaspect, the dose amount and/or frequency of administration of the antibody is modulated to reduce the concentration of the antibody to which the red blood cells are exposed. In another embodimentaspect, the antibody is modified to comprise pH-sensitive binding to the BBB-R.

In another embodimentaspect, a further compound is administered in addition to the antibody and the coupled compound. In one such embodimentaspect, the further compound is responsible for or contributes to the lack of reduction of reticulocyte levels. In another such embodimentaspect, the further compound inhibits or prevents the activation or activity of the complement pathway (see, e.g., Mollnes and Kirschfink (2006) Molec. Immunol. 43:107-121). In another such embodimentaspect, the further compound protects reticulocytes from antibody-related depletion. In another such embodimentaspect, the further compound supports the growth, development, or reestablishment of reticulocytes. In another embodimentaspect, the further compound is selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid and vitamin B12. In another embodimentaspect, the further compound is red blood cells or reticulocytes from the same subject. In another embodimentaspect, the further compound is red blood cells or reticulocytes from another subject.

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

increase in CNS retention results in a ratio of greater than 0.1% at one or more time points after administration. In another embodimentaspect, 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 embodimentaspect, the increase in CNS retention of the compound is measured by imaging. In another embodimentaspect, 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 embodimentaspect, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such embodimentaspect, the IC50 is from about 5 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 50 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 100 nM to about 100μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 ntibody. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such

embodimentaspect, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such embodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA.

In another embodimentaspect, the compound-coupled antibody is administered at a therapeutic dose. In one such embodimentaspect, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodimentaspect, the compound-coupled antibody is administered at a dose and dose frequency that minimizes red blood cell interaction with the compound-coupled antibody while still facilitating compound delivery across the BBB into the CNS at therapeutic levels.

In another embodimentaspect, the compound is covalently coupled to the antibody. In one such embodimentaspect, the compound is joined to the antibody by a linker. In one such embodimentaspect, the linker is cleavable. In another such embodimentaspect, the linker is not cleavable. In another such embodimentaspect, the compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly

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

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

In ~~another embodiment, the invention~~one aspect, there is provided~~s~~ 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, wherein reduction of red blood cell levels in the subject upon compound-coupled antibody administration to the subject is decreased or eliminated. In one embodiment~~aspect~~, 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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodiment~~aspect~~, the BBB-R is a human BBB-R. In one such embodiment~~aspect~~, the BBB-R is TfR. In another such embodiment~~aspect~~, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodiment~~aspect~~, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodiment~~aspect~~, the red blood cells are immature red blood cells. In another such embodiment~~aspect~~, the immature red blood cells are reticulocytes. In another embodiment~~aspect~~, reduction of reticulocyte levels is accompanied by acute clinical symptoms. In another embodiment~~aspect~~, the method further comprises the step of monitoring the subject for depletion of red blood cells.

In another embodiment~~aspect~~, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodiment~~aspect~~, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodiment~~aspect~~, the effector function of the antibody Fc region is modified. In one such embodiment~~aspect~~, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In another such embodiment~~aspect~~, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodiment~~aspect~~, the glycosylation of the antibody is reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodiment~~aspect~~, the antibody is produced in a non-mammalian cell

production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the Fc region and/or the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, complement-triggering 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 that engages the complement pathway. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody. In another such embodimentaspect, the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the CH1 region to impair binding to C3. In one such embodimentaspect, the point mutation is at position 132 (see, e.g., Vidarte et al., (2001) J. Biol. Chem. 276(41): 38217-38223).

In another embodimentaspect, the dose amount and/or frequency of administration of the antibody is modulated to reduce the concentration of the antibody to which the red blood cells are exposed. In another embodimentaspect, the antibody is modified to comprise pH-sensitive binding to the BBB-R.

In another embodimentaspect, a further compound is administered in addition to the antibody and the coupled compound. In one such embodimentaspect, the further compound is responsible for or contributes to the lack of reduction of reticulocyte levels. In another such embodimentaspect, the further compound inhibits or prevents the activation or activity of the complement pathway (see, e.g., Mollnes and Kirschfink (2006) Molec. Immunol. 43:107-121). In another such embodimentaspect, the further compound protects reticulocytes from antibody-related depletion. In another such embodimentaspect, the further compound supports the growth, development, or reestablishment of reticulocytes. In another embodimentaspect, the further compound is selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid and vitamin B12. In another embodimentaspect, the further compound is red blood cells or reticulocytes from the same subject. In another embodimentaspect, the further compound is red blood cells or reticulocytes from another subject.

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

In one embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such embodimentaspect, the IC50 is from about 5 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 50 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 100 nM to about 100μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such aspectembodiment, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such mbodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody

specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^{D}/BACE1 antibody and the anti-TfR^{E}/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA.

In another embodimentaspect, the compound-coupled antibody is administered at a therapeutic dose. In one such embodimentaspect, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodimentaspect, the compound-coupled antibody is administered at a dose and dose frequency that minimizes red blood cell interaction with the compound-coupled antibody while still facilitating compound delivery across the BBB into the CNS at therapeutic levels.

In another embodimentaspect, the compound is covalently coupled to the antibody. In one such embodimentaspect, the compound is joined to the antibody by a linker. In one such embodimentaspect, the linker is cleavable. In another such embodimentaspect, the linker is not cleavable. In another such embodimentaspect, the compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

In one aspect, there is provided use of 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 the manufacture of a medicament for treatment of a neurological disorder in a mammal, wherein reduction of red blood cell levels in the subject upon compound-coupled antibody administration to the subject is decreased or eliminated.

In another embodiment the disclosureinvention 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, and wherein reduction of red blood cell levels in the subject upon compound-coupled antibody administration to the subject is decreased or eliminated. In one embodimentaspect, 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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodimentaspect, the BBB-R is a human BBB-R. In one such embodimentaspect, the BBB-R is TfR. In another such embodimentaspect, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodimentaspect, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodimentaspect, the red blood cells are immature red blood cells. In another such embodimentaspect, the immature red blood cells are reticulocytes. In another embodimentaspect, reduction of reticulocyte levels is accompanied by acute clinical symptoms. In another embodimentaspect, the method further comprises the step of monitoring the subject for depletion of red blood cells.

In another embodimentaspect, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodimentaspect, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodimentaspect, the effector function of the antibody Fc region is modified. In one such embodimentaspect, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In another such embodimentaspect, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodimentaspect, the antibody is produced in a non-mammalian cell production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is

reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the dose amount and/or frequency of administration of the antibody is modulated to reduce the concentration of the antibody to which the red blood cells are exposed. In another embodimentaspect, the antibody is modified to comprise pH-sensitive binding to the BBB-R.

In another embodimentaspect, a further compound is administered in addition to the antibody and the coupled compound. In one such embodimentaspect, the further compound is responsible for or contributes to the lack of reduction of reticulocyte levels. In another such embodimentaspect, the further compound inhibits or prevents the activation or activity of the complement pathway (see, e.g., Mollnes and Kirschfink (2006) Molec. Immunol. 43:107-121). In another such embodimentaspect, the further compound protects reticulocytes from antibody-related depletion. In another such embodimentaspect, the further compound supports the growth, development, or reestablishment of reticulocytes. In another embodimentaspect, the further compound is selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid and vitamin B12. In another embodimentaspect, the further compound is red blood cells or reticulocytes from the same subject. In another embodimentaspect, the further compound is red blood cells or reticulocytes from another subject.

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

disorder. In another such embodimentaspect, 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 embodimentaspect, the treating results in lessening or elimination of disorder symptoms. In another embodimentaspect, the treating results in amelioration of the neurological disorder.

In another embodimentaspect, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such embodimentaspect, the IC50 is from about 5 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 50 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 100 nM to about 100μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such

embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such embodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA.

In another embodimentaspect, the compound-coupled antibody is administered at a therapeutic dose. In one such embodimentaspect, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodimentaspect, the compound-coupled antibody is administered at a dose and dose frequency that minimizes red blood cell interaction with the compound-coupled antibody while still facilitating compound delivery across the BBB into the CNS at therapeutic levels.

In another embodimentaspect, the compound is covalently coupled to the antibody. In one such embodimentaspect, the compound is joined to the antibody by a linker. In one such embodimentaspect, the linker is cleavable. In another such embodimentaspect, the linker is not cleavable. In another such embodimentaspect, the compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such

embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

In another embodiment, the inventionone aspect, there is provideds a method of improving the safety in a subject of an antibody that binds a BBB-R comprising modifying one or more properties of the antibody such that administration of the antibody decreases or eliminates reduction of red blood cell levels in the subject observed upon administration of the unmodified antibody. In one embodimentaspect, 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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodimentaspect, the BBB-R is a human BBB-R. In one such embodimentaspect, the BBB-R is TfR. In another such embodimentaspect, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodimentaspect, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodimentaspect, the red blood cells are immature red blood cells. In another such embodimentaspect, the immature red blood cells are reticulocytes. In another embodimentaspect, reduction of reticulocyte levels is accompanied by acute clinical symptoms.

In another embodimentaspect, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodimentaspect, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodimentaspect, the modification of the affinity of the antibody is measured relative to a wild-type antibody of the same isotype not having modified (i.e., decreased) affinity for the BBB-R. In another such embodimentaspect, the effector function of the antibody Fc region is modified. In one such embodimentaspect, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In another such embodimentaspect, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodimentaspect, the antibody is produced in a non-mammalian cell production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups

already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the Fc region and/or the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, complement-triggering 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 that engages the complement pathway. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody. In another such embodimentaspect, the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the CH1 region to impair binding to C3. In one such embodimentaspect, the point mutation is at position 132 (see, e.g., Vidarte et al., (2001) J. Biol. Chem. 276(41): 38217-38223).

In another embodimentaspect, the dose amount and/or frequency of administration of the antibody is modulated to reduce the concentration of the antibody to which the red blood cells are

exposed. In another embodimentaspect, the antibody is modified to comprise pH-sensitive binding to the BBB-R.

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

In another embodimentaspect, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such embodimentaspect, the IC50 is from about 5 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 50 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 100 nM to about 100μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is

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

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such embodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA.

In another embodimentaspect, the antibody is selected from a panel of antibodies based upon the affinity of the selected antibody. In another embodimentaspect, the antibody is engineered to have the desired affinity. In one such embodimentaspect, 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 another embodimentaspect, the compound-coupled antibody is administered at a therapeutic dose. In one such embodimentaspect, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodimentaspect, the compound-coupled antibody is administered at a dose and dose frequency that minimizes red blood cell interaction with the compound-coupled antibody while still facilitating compound delivery across the BBB into the CNS at therapeutic levels.

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

compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

In ~~another embodiment, the invention~~one aspect, there is provideds a method of making an antibody useful for transporting a compound across the BBB with improved safety comprising selecting an antibody specific for a blood-brain barrier receptor (BBB-R) that has a desirably low affinity for the BBB-R, and modifying one or more properties of the antibody such that administration of the antibody decreases or eliminates reduction of red blood cell levels in the subject observed upon administration of an unmodified antibody. In one embodimentaspect, 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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodimentaspect, the BBB-R is a human BBB-R. In one such embodimentaspect, the BBB-R is TfR. In another such embodimentaspect, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodimentaspect, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodimentaspect, the red blood cells are immature red blood cells. In another such embodimentaspect, the immature red blood cells are reticulocytes. In another embodimentaspect, reduction of reticulocyte levels is accompanied by acute clinical symptoms.

In another embodimentaspect, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodimentaspect, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodimentaspect, the modification of the affinity of the antibody is measured relative to a wild-type antibody of the same isotype not having modified (i.e., decreased) affinity for the BBB-R. In another such embodimentaspect, the effector function of the antibody Fc region is modified. In one such embodimentaspect, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In another such embodimentaspect, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodimentaspect, the antibody is produced in a non-mammalian cell production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the Fc region and/or the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such

embodimentaspect, the modification is a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, complement-triggering 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 that engages the complement pathway. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody. In another such embodimentaspect, the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the CH1 region to impair binding to C3. In one such embodimentaspect, the point mutation is at position 132 (see, e.g., Vidarte et al., (2001) J. Biol. Chem. 276(41): 38217-38223).

In another embodimentaspect, the dose amount and/or frequency of administration of the antibody is modulated to reduce the concentration of the antibody to which the red blood cells are exposed. In another embodimentaspect, the antibody is modified to comprise pH-sensitive binding to the BBB-R.

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

In another embodimentaspect, the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM. In another such embodimentaspect, the IC50 is from about 5 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 50 nM to about 100μM. In another such embodimentaspect, the IC50 is from about 100 nM to about 100μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM. In another embodimentaspect, the antibody has an affinity for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such embodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA.

In another embodimentaspect, the antibody is selected from a panel of antibodies based upon the affinity of the selected antibody. In another embodimentaspect, the antibody is engineered to have the desired affinity. In one such embodimentaspect, 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 another embodimentaspect, the compound-coupled antibody is administered at a therapeutic dose. In one such embodimentaspect, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodimentaspect, the compound-coupled antibody is administered at a dose and dose frequency that minimizes red blood cell interaction with the compound-coupled antibody while still facilitating compound delivery across the BBB into the CNS at therapeutic levels.

In another embodimentaspect, the compound is covalently coupled to the antibody. In one such embodimentaspect, the compound is joined to the antibody by a linker. In one such embodimentaspect, the linker is cleavable. In another such embodimentaspect, the linker is not cleavable. In another such embodimentaspect, the compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

In one aspect, there is provided an antibody which binds to a BBB-R, wherein the affinity of the antibody for the BBB-R is from about 5 nM to about 50 μM or the dissociation half-life of the antibody for the BBB-R is from about 30 seconds to about 2 minutes, and wherein one or more properties of the antibody have been modified to reduce at least one undesired side effect on red blood cells.

In another embodiment, the disclosureinvention 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 50 μM, and wherein one or more properties of the antibody have been

modified to reduce at least one undesired side effect on red blood cells. In one embodimentaspect, 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), glucose transporter 1 (Glut1) and heparin-binding epidermal growth factor-like growth factor (HB-EGF). In another such embodimentaspect, the BBB-R is a human BBB-R. In one such embodimentaspect, the BBB-R is TfR. In another such embodimentaspect, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodimentaspect, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

In another embodimentaspect, the red blood cells are immature red blood cells. In another such embodimentaspect, the immature red blood cells are reticulocytes. In another embodimentaspect, reduction of reticulocyte levels is accompanied by acute clinical symptoms.

In another embodimentaspect, one or more properties of the antibody have been modified to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in the subject. In one such embodimentaspect, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodimentaspect, the modification of the affinity of the antibody is measured relative to a wild-type antibody of the same isotype not having modified (i.e., decreased) affinity for the BBB-R. In another such embodimentaspect, the effector function of the antibody Fc region is modified. In one such embodimentaspect, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In another such embodimentaspect, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by production of the antibody in an environment that does not permit wild-type glycosylation. In one such embodimentaspect, the antibody is produced in a non-mammalian cell production system. In another such embodimentaspect, the antibody is produced synthetically. In another such embodimentaspect, the glycosylation of the antibody is reduced by removal of carbohydrate groups already present on the antibody. In another such embodimentaspect, the glycosylation of the antibody is reduced by modification of the antibody such that wild-type glycosylation does not occur. In another such embodimentaspect, 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. In another embodimentaspect, the effector function is reduced or eliminated by modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function.

In another embodimentaspect, the Fc region is modified to reduce or eliminate effector function. In one such embodimentaspect, the effector function is reduced or eliminated by at least one modification of the Fc region. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, the effector 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 competent for effector function. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody.

In another embodimentaspect, the Fc region and/or the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321. In another such embodimentaspect, the modification is elimination of some or all of the Fc region. In another such embodimentaspect, complement-triggering 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 that engages the complement pathway. In one such embodimentaspect, the antibody is selected from a Fab or a single chain antibody. In another such embodimentaspect, the non-Fc region of the antibody is modified to reduce or eliminate activation of the complement pathway by the antibody. In one such embodimentaspect, the modification is a point mutation of the CH1 region to impair binding to C3. In one such embodimentaspect, the point mutation is at position 132 (see, e.g., Vidarte et al., (2001) J. Biol. Chem. 276(41): 38217-38223).

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

In another embodimentaspect, the antibody has an IC50 for the BBB-R from about 30 nM to about 30 μM. In another such embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, 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 embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using scatchard analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the affinity of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition ELISA.

In another embodimentaspect, the dissociation half-life of the antibody from the BBB-R to which it specifically binds is from about 30 seconds to about 30 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 20 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 10 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 5 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 3 minutes. In another such embodimentaspect, the dissociation half-life is from about 30 seconds to about 2 minutes. In another such embodimentaspect, the dissociation half-life is about two minutes. In another such embodimentaspect, the dissociation half-life is one minute or less. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^A/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another such embodimentaspect, the compound-coupled antibody specifically binds to TfR and has a dissociation half-life for TfR between those dissociation half-lives observed for the anti-TfR^D/BACE1 antibody and the anti-TfR^E/BACE1 antibody from their respective binding to TfR. In another embodimentaspect, the dissociation half-life of the anti-

BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodimentaspect, the dissociation half-life of the anti-BBB-R or anti-BBB-R/compound for the BBB-R is measured using a competition binding assay, such as a competition ELISA.

In another embodimentaspect, the antibody is selected from a panel of antibodies based upon the affinity of the selected antibody. In another embodimentaspect, the antibody is engineered to have the desired affinity. In one such embodimentaspect, 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 another embodimentaspect, a compound is covalently coupled to the antibody. In one such embodimentaspect, the compound is joined to the antibody by a linker. In one such embodimentaspect, the linker is cleavable. In another such embodimentaspect, the linker is not cleavable. In another such embodimentaspect, the compound is directly linked to the antibody. In one such embodimentaspect, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodimentaspect, 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 embodimentaspect, 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), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodimentaspect, the multispecific antibody binds both TfR and BACE1. In another such embodimentaspect, the multispecific antibody binds both TfR and Abeta. In another such embodimentaspect, the multispecific antibody is labeled. In another embodimentaspect, the compound is reversibly coupled to the antibody such that the compound is released from the antibody concurrent with or after BBB transport.

It will be appreciated that any of the foregoing aspects may be applied singly or in combination with the foregoing embodiment.

In one aspect, there is provided use of an antibody that binds with low affinity to a BBB-R and that does not impact red blood cell levels for the manufacture of a medicament for treating a neurological disorder.

In one aspect, there is provided use of the antibody as described herein for the manufacture of a medicament for treating a neurological disorder.

In another embodiment, the disclosureinvention provides the use of an antibody that binds with low affinity to a BBB-R and that does not reduce red blood cell levels for the manufacture of a medicament for treating a neurological disorder. 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.

In one aspect, there is provided an antibody that binds with low affinity to a BBB-R and which does not impact red blood cell levels for use in treating a neurological disorder.
In one aspect, there is provided an antibody as described herein for use in treating a neurological disorder.

In another embodiment, the disclosure~~invention~~ provides an antibody that binds with low affinity to a BBB-R and that does not reduce red blood cell levels for use in treating a neurological disorder. 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.

In another embodiment, the disclosure~~invention~~ 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, wherein the antibody does not reduce red blood cell levels.

The disclosure~~invention~~ 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, and wherein administration of the antibody does not decrease red blood cell levels.

In one aspect, there is provided use of an anti-TfR antibody comprising at least partial effector function in the manufacture of a medicament for treating a disease or disorder associated with or caused by elevated red blood cell levels.

The disclosure~~invention~~ additionally provides a method of treating a disease or disorder associated with or caused by elevated red blood cell levels in a subject comprising administering an anti-TfR antibody comprising at least partial effector function to the subject. In one embodiment~~aspect~~, the administering step is at a dose and/or dose frequency calibrated to minimize acute clinical symptoms of the antibody administration.

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

BRIEF DESCRIPTION OF THE FIGURES

Figures 1A-1E depict the results of experiments assessing the affinities of anti-transferrin receptor ("TfR") and anti-TfR/beta-secretase ("BACE1") variants for TfR, as well as concentrations of the antibody and $A\beta_{1-40}$ after administration in mice, as described in Example 1. The competitive ELISA assay results in Figure 1A show that anti-TfR/BACE1 variants and anti-TfR variants have distinct affinities for TfR. Figures 1B and 1D show, respectively, mean serum and brain antibody concentrations in wild-type mice after a single 50 mg/kg intravenous injection of control IgG, anti-BACE1, or an anti-TfR/BACE1 variant (n= 6 per group). Figures 1C and 1E show, respectively, plasma and brain concentrations of $A\beta_{1-40}$ in these same treated mice, as a marker of the activity of the injected antibody.