immature reticulocyte populations without impacting normal transferrin transport into the CNS. Dosing of such an antibody could be modulated such that acute clinical symptoms could be minimized (ie, by dosing at a very low dose or at widely-spaced intervals), as well-understood in the art.

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

Accordingly, in one aspect there is provided use of an antibody which binds to a transferrin receptor (TfR) in the manufacture of a medicament for transporting a compound across the blood-brain barrier, wherein the antibody is coupled to the compound, and wherein the antibody binds TfR with low affinity and wherein 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 a subject such that reduction of red blood cell levels in the subject upon antibody administration is decreased or eliminated.

Disclosed is 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 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, the BBB-R is a human BBB-R. In one such embodiment, the BBB-R is TfR. In another such embodiment, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

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

In another embodiment, 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, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodiment, the effector function of the antibody Fc region is modified. In one such embodiment, the effector function has been reduced or eliminated relative to the effector function of a wild-type antibody of the same isotype. In

another embodiment, 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 embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such aspectembodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodiment, 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 embodiment, the compound is covalently coupled to the antibody. In one such embodiment, the compound is joined to the antibody by a linker. In one such embodiment, the linker is cleavable. In another such embodiment, the linker is not cleavable. In another such embodiment, the compound is directly linked to the antibody. In one such embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such embodiment, the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodiment, the multispecific antibody binds both TfR and BACE1. In another such embodiment, the multispecific antibody binds both TfR and Abeta. In another such embodiment, the multispecific antibody is labeled. In another embodiment, 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~~Disclosed is 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 embodiment, the BBB-R is selected from the group consisting of transferrin receptor (TfR), insulin receptor,

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 embodiment, the compound is covalently coupled to the antibody. In one such embodiment, the compound is joined to the antibody by a linker. In one such embodiment, the linker is cleavable. In another such embodiment, the linker is not cleavable. In another such embodiment, the compound is directly linked to the antibody. In one such embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such embodiment, the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodiment, the multispecific antibody binds both TfR and BACE1. In another such embodiment, the multispecific antibody binds both TfR and Abeta. In another such embodiment, the multispecific antibody is labeled. In another embodiment, 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~~Disclosed is 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 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, the BBB-R is a human BBB-R. In one such embodiment, the BBB-R is TfR. In another such embodiment, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another

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 embodiment, the multispecific antibody binds both TfR and BACE1. In another such embodiment, the multispecific antibody binds both TfR and Abeta. In another such embodiment, the multispecific antibody is labeled. In another embodiment, 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~~Disclosed is 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 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, the BBB-R is a human BBB-R. In one such embodiment, the BBB-R is TfR. In another such embodiment, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

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

In another embodiment, 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, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodiment, the effector function of the antibody Fc region is modified. In one such embodiment, 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, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodiment, the glycosylation of the antibody is

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~~Disclosed is 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, 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, the BBB-R is a human BBB-R. In one such embodiment, the BBB-R is TfR. In another such embodiment, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

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

In another embodiment, 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, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodiment, the effector function of the antibody Fc region is modified. In one such embodiment, 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, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodiment, 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, the antibody is produced in a non-mammalian cell

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 embodiment, 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 embodiment, 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 embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such embodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodiment, 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 embodiment, the compound is covalently coupled to the antibody. In one such embodiment, the compound is joined to the antibody by a linker. In one such embodiment, the linker is cleavable. In another such embodiment, the linker is not cleavable. In another such embodiment, the compound is directly linked to the antibody. In one such embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such embodiment, the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodiment, the multispecific antibody binds both TfR and BACE1. In another such embodiment, the multispecific antibody binds both TfR and Abeta. In another such embodiment, the multispecific antibody is labeled. In another embodiment, 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 disclosure provides a method of treating a neurological disorder in a mammal comprising treating the mammal with an antibody that binds a BBB-R

embodiment, the multispecific antibody is labeled. In another embodiment, 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~~Disclosed is 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 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, the BBB-R is a human BBB-R. In one such embodiment, the BBB-R is TfR. In another such embodiment, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

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

In another embodiment, 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, the affinity of the antibody for the BBB-R is modified, i.e., decreased. In another such embodiment, 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 embodiment, the effector function of the antibody Fc region is modified. In one such embodiment, 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, the effector function is reduced or eliminated by reduction of glycosylation of the antibody. In another such embodiment, 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, the antibody is produced in a non-mammalian cell production system. In another such embodiment, the antibody is produced synthetically. In another such embodiment, the glycosylation of the antibody is reduced by removal of carbohydrate groups

compound is directly linked to the antibody. In one such embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such embodiment, the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodiment, the multispecific antibody binds both TfR and BACE1. In another such embodiment, the multispecific antibody binds both TfR and Abeta. In another such embodiment, the multispecific antibody is labeled. In another embodiment, 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 a method of making an antibody useful for transporting a compound across the BBB with improved safety comprising selecting an antibody specific for a transferrin receptor (TfR) that has low affinity for the TfR, and modifying one or more properties of the antibody to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in a subject such that reduction of red blood cell levels in the subject upon antibody administration is decreased or eliminated compared to the unmodified antibody. Also disclosed is 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 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, the BBB-R is a human BBB-R. In one such embodiment, the BBB-R is TfR. In another such embodiment, the BBB-R is TfR, and the antibody does not inhibit TfR activity. In another such embodiment, the BBB-R is TfR and the antibody does not inhibit the binding of TfR to transferrin.

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

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

In another embodiment, the compound-coupled antibody is administered at a therapeutic dose. In one such embodiment, the therapeutic dose is a dose that saturates the BBB-R to which the antibody specifically binds. In another such embodiment, 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 embodiment, the compound is covalently coupled to the antibody. In one such embodiment, the compound is joined to the antibody by a linker. In one such embodiment, the linker is cleavable. In another such embodiment, the linker is not cleavable. In another such embodiment, the compound is directly linked to the antibody. In one such embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such embodiment, the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodiment, the multispecific antibody binds both TfR and BACE1. In another such embodiment, the multispecific antibody binds both TfR and Abeta. In another such embodiment, the multispecific antibody is labeled. In another embodiment, 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 TfR, wherein the affinity of the antibody for the TfR is from about 5 nM to about 50 μM or the dissociation half-life of the antibody for the TfR 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~~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 disclosure provides an antibody which binds to a blood-brain barrier receptor (BBB-R), wherein the affinity of the antibody for the BBB-R is from about 5 nM to about 50 μM, and wherein one or more properties of the antibody have been

BBB-R or anti-BBB-R/compound for the BBB-R is measured using BIACORE analysis. In another embodiment, 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 embodiment, the antibody is selected from a panel of antibodies based upon the affinity of the selected antibody. In another embodiment, the antibody is engineered to have the desired affinity. In one such embodiment, the antibody is generated using any art-known protein engineering methodology including, but not limited to, phage display, yeast display, random mutagenesis, and site-directed mutagenesis.

In another embodiment, a compound is covalently coupled to the antibody. In one such embodiment, the compound is joined to the antibody by a linker. In one such embodiment, the linker is cleavable. In another such embodiment, the linker is not cleavable. In another such embodiment, the compound is directly linked to the antibody. In one such embodiment, the antibody is a multispecific antibody and the compound forms one portion of the multispecific antibody. In another such embodiment, the multispecific antibody comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen. In another such embodiment, the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), Tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), interleukin 6 receptor (IL6R), TNF receptor 1 (TNFR1), interleukin 1 beta (IL1β), and caspase 6. In another such embodiment, the multispecific antibody binds both TfR and BACE1. In another such embodiment, the multispecific antibody binds both TfR and Abeta. In another such embodiment, the multispecific antibody is labeled. In another embodiment, 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~~TfR 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 disclosure 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~~TfR 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 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 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 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 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, 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 may be combined with one another and/or with the further aspects and embodiments of the disclosure 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.

WHAT IS CLAIMED IS:

1. 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.

2. 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.

3. 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.

4. 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.

5. A method of optimizing the pharmcokinetics and/or pharmacodynamics of a compound to be efficacious in the CNS in 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.

6. 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.

7. The method of any of claims 1-5 or the use of claim 6, wherein 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),wherein:

i) optionally the BBB-R is TfR, or

ii) optionally the red blood cells are immature red blood cells, or

iii) optionally the immature red blood cells are reticulocytes, or

iv) optionally reduction of reticulocyte levels is accompanied by acute clinical symptoms, or

v) optionally 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 or mammal, or

vi) optionally the one or more properties are selected from the effector function of the antibody Fc region, the complement activation function of the antibody and the affinity of the antibody for the BBB-R, or

vii) optionally the one or more properties are selected from the effector function of the antibody Fc region and the complement activation function of the antibody, and wherein the effector function or complement activation function has been reduced or eliminated relative to a wild-type antibody of the same isotype, or

viii) optionally the effector function is reduced or eliminated by a method selected from reduction of glycosylation of the antibody, modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function, and modification of the Fc region, or

ix) optionally the glycosylation of the antibody is reduced by a method selected from: production of the antibody in an environment that does not permit wild-type glycosylation; removal of carbohydrate groups already present on the antibody; and modification of the antibody such that wild-type glycosylation does not occur, or

x) optionally the antibody is produced in a non-mammalian cell production system, or where the antibody is produced synthetically, or

xi) optionally 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.

8. The method of claim 7viii), wherein optionally the effector function is reduced or eliminated by at least one modification of the Fc region, or
wherein optionally the effector function or complement activation function is reduced or eliminated by deletion of all or a portion of the Fc region, or by engineering the antibody such that it does not include an Fc region or non-Fc region competent for effector function or complement activation function, or
wherein the modification is selected from: a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 238, 239, 248, 249, 252, 254, 265, 268, 269, 270, 272, 278, 289, 292, 293, 294, 295, 296, 297, 298, 301, 303, 322, 324, 327, 329, 333, 335, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, and 439; a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321; eliminating some or all of the Fc region, and a point mutation at position 132 of the CH1 domain.

9. The method of claim 7iv), wherein: i) the dose amount and/or frequency of administration is modulated to reduce the concentration of antibody to which the red blood cells are exposed, or
ii) the antibody is modified to comprise pH-sensitive binding to the BBB-R, or
iii) a further compound is to be administered in addition to the antibody.

10. The method of claim 9iii), wherein i) the further compound is responsible for or contributes to the lack of reduction of reticulocyte levels, or
ii) optionally the further compound protects reticulocytes from antibody-related depletion or supports the growth, development, or reestablishment of reticulocytes.

11. The method of claim 10ii), wherein the further compound is selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid, and vitamin B12, or
wherein the further compound is red blood cells or reticulocytes from the same or another subject.

12. The method of claim 7vi), wherein the affinity of the antibody for the BBB-R is further decreased.

13. The method of any of claims 1-12, further comprising the step of monitoring the subject for depletion of red blood cells.

14. The method of any of claims 1-13, wherein: i) the compound is a neurological disorder drug or an imaging agent, or ii) the antibody does not impair the binding of the BBB-R to one or more of its native ligands, or iii) the blood-brain barrier is in a mammal, or iv) the antibody has an IC50 for the BBB-R from about 1 nM to about 100 μM, or v) the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM, or vi) the antibody coupled to the compound has an affinity for the BBB-R from about 30 nM to about 30 μM, or vii) the antibody coupled to the compound has an affinity for the BBB-R from about 30 nM to about 1 μM, or viii) the antibody coupled to the compound has a dissociation half-life for the BBB-R from about 30 seconds to about 5 minutes, or from about 30 seconds to about 2 minutes, or ix) the antibody coupled to the compound is to be administered at a therapeutic dose, and or x) the antibody is a multispecific antibody and the compound optionally forms one portion of the multispecific antibody.

15. The method of any of claims 2-13, wherein the increase or decrease is measured relative to a wild-type antibody of the same isotype not having lowered affinity for the BBB-R.

16. The method of claim 14iii), wherein the mammal has a neurological disorder, wherein optionally 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.

17. The method of claim 14iii) wherein the mammal is a human.

18. The method of claim 14iv), wherein the IC50 is from about 5 nM to about 100μM, or wherein the IC50 is from about 50 nM to about 100μM, or wherein the IC50 is from about 100 nM to about 100μM.

19. The method of claim 7i), wherein the antibody does not inhibit the binding of TfR to transferrin.

20. The method of claim 14ix), wherein the therapeutic dose is BBB-R-saturating.

21. The method of claim 14x) wherein 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,

wherein optionally the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2),

parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6, or wherein optionally the multispecific antibody binds both TfR and BACE1, or wherein the multispecific antibody binds both TfR and Abeta.

22. 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.

23. 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 the unmodified antibody.

24. The method of claims 22 or 23 wherein 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), wherein:

i) optionally the BBB-R is transferrin receptor (TfR), or

ii) optionally the red blood cells are reticulocytes, or

iii) optionally reduction of reticulocyte levels is accompanied by acute clinical symptoms, or

iv) optionally the one or more properties is selected from the effector function of the antibody Fc region, the complement activation function of the antibody and the affinity of the antibody for the BBB-R, or

v) optionally the one or more properties is selected from the effector function of the antibody Fc region and the complement activation function of the antibody, and wherein the effector function or the complement activation function has been reduced or eliminated relative to a wild-type antibody of the same isotype, or

vi) optionally the effector function is reduced or eliminated by a method selected from reduction of glycosylation of the antibody, modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function, and modification of the Fc region, or

vii) optionally the glycosylation of the antibody is reduced by a method selected from: production of the antibody in an environment that does not permit wild-type glycosylation; removal of carbohydrate groups already present on the antibody; and modification of the antibody such that wild-type glycosylation does not occur, or

viii) optionally wherein the antibody is produced in a non-mammalian cell production system, or where the antibody is produced synthetically, or

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

25. The method of claim 24vi), wherein the effector function or the complement activation function is reduced or eliminated by at least one modification of the Fc region or the non-Fc region of the antibody, wherein:

i) optionally the effector function or complement activation function is reduced or eliminated by deletion of all or a portion of the Fc region, or by engineering the antibody such that it does not include an Fc region competent for effector function or complement activation function, or

ii) the modification is selected from: a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, and 439; a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321, eliminating some or all of the Fc region, and a point mutation at position 132 of the CH1 domain.

26. The method of claim 24iii), wherein the dose amount and/or frequency of administration is modulated to reduce the concentration of antibody to which the red blood cells are exposed, or

wherein the antibody is modified to comprise pH-sensitive binding to the BBB-R.

27. The method of claim 24iv), wherein the affinity of the antibody for the BBB-R is further decreased,

wherein optionally the decrease is measured relative to a wild-type antibody of the same isotype not having lowered affinity for the BBB-R.

28. The method of claims 22 or 23, wherein: i) the affinity for the BBB-R is from about 1 nM to about 100 μM, or ii) the antibody is selected from a panel of antibodies based upon the affinity of the selected antibody, or iii) the antibody is engineered to have the

affinity, or iv) the antibody is coupled with a therapeutic compound, or v) the antibody is a multispecific antibody which comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen.

29. The method of claim 28i), wherein the antibody has an IC50 of from about 1nM to about 100 μM, or
wherein the IC50 is from about 5 nM to about 100 μM, or
wherein the IC50 is from about 50 nM to about 100 μM, or
wherein the IC50 is from about 100 nM to about 100 μM.

30. The method of any of claims 22-27, wherein the antibody has an affinity for the BBB-R from about 5 nM to about 50 μM, or
wherein the antibody has a dissociation half-life for the BBB-R from about 30 seconds to about 5 minutes, or from about 30 seconds to about 2 minutes.

31. The method of claim 28iv) wherein: i) the therapeutic compound is a neurological disorder drug, or
ii) the antibody coupled to the compound has an affinity for the BBB-R from about 30 nM to about 30 μM.

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

33. The method of claim 28v), wherein the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6.

34. The method of claim 33, wherein (i) the multispecific antibody binds both TfR and BACE1, or
(ii) the multispecific antibody binds both TfR and Abeta.

35. The method of any of claims 22-34i), wherein the antibody does not impair the binding of the BBB-R to one or more of its native ligands,
wherein optionally the antibody does not inhibit the binding of TfR to transferrin.

36. 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.

37. The antibody of claim 36 wherein i) 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), wherein:
ii) optionally the BBB-R is transferrin receptor (TfR), or
iii) optionally the red blood cells are reticulocytes, or
iv) optionally at least one undesired side effect on red blood cells is selected from reduction of reticulocyte levels and acute clinical symptoms, or
v) optionally one or more properties is selected from the effector function of the antibody Fc region, the complement activation function of the antibody and the affinity of the antibody for the BBB-R, or
vi) optionally one or more properties is selected from the effector function of the antibody Fc region and the complement activation function of the antibody, and wherein the effector function or complement activation function has been reduced or eliminated relative to a wild-type antibody of the same isotype, or
vii) optionally the effector function is reduced or eliminated by a method selected from reduction of glycosylation of the antibody, modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function, and modification of the Fc region, or
viii) optionally the glycosylation of the antibody is reduced by a method selected from: production of the antibody in an environment that does not permit wild-type glycosylation; removal of carbohydrate groups already present on the antibody; and modification of the antibody such that wild-type glycosylation does not occur, or
ix) optionally wherein the antibody is produced in a non-mammalian cell production system, or where the antibody is produced synthetically, or
x) wherein the Fc region of the antibody comprises a mutation at position 297 such that the wild-type asparagine residue at that position is replaced with another amino acid that interferes with glycosylation at that position.

38. The antibody of claim 37vii), wherein the effector function or complement activation function is reduced or eliminated by at least one modification of the Fc region or the non-Fc region,

wherein optionally the effector function or complement activation function is reduced or eliminated by deletion of all or a portion of the Fc region, or by engineering the antibody such that it does not include an Fc region competent for effector function, or

wherein the modification is selected from: a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, and 439; a point mutation of the Fc region to impair binding to C1q selected from the following positions: 270, 322, 329, and 321; eliminating some or all of the Fc region, and a point mutation at position 132 of the CH1 domain.

39. The antibody of claim 37v), wherein: i) the antibody is modified to comprise pH-sensitive binding to the BBB-R, or

ii) the affinity of the antibody for the BBB-R is further decreased.

40. The antibody of claim 39ii), wherein the decrease is measured relative to a wild-type antibody of the same isotype not having lowered affinity for the BBB-R.

41. The antibody of claim 36, wherein: i) the affinity is measured as an IC50, or

ii) the antibody is selected from a panel of antibodies based upon the affinity of the selected antibody, or

iii) the antibody is engineered to have the affinity, or

iv) the antibody is further coupled with a therapeutic compound.

42. The antibody of claim 41iv) wherein the therapeutic compound is a neurological disorder drug, or

wherein the antibody coupled to the compound has an affinity for the BBB-R from about 30 nM to about 30 μM, or

wherein the antibody is a multispecific antibody and the compound optionally forms one portion of the multispecific antibody.

43. The antibody of claim 36 wherein the antibody is a multispecific antibody which comprises a first antigen binding site which binds the BBB-R and a second antigen binding site which binds a brain antigen.

44. The antibody of claim 43, wherein the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6

(DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6.

45. The antibody of claim 44, wherein the multispecific antibody binds both TfR and BACE1, or

wherein the multispecific antibody binds both TfR and Abeta.

46. The antibody of any of claims 36-45, wherein the antibody does not impair the binding of the BBB-R to one or more of its native ligands.

47. The antibody of claim 46, wherein the antibody does not inhibit the binding of TfR to transferrin.

48. 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.

49. Use of the antibody of any of claims 36-47 for the manufacture of a medicament for treating a neurological disorder.

50. 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.

51. An antibody of any of claims 36-47 for use in treating a neurological disorder.

52. 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.

53. The use of claim 52, wherein the antibody is to be administered at a dose and/or dose frequency calibrated to minimize acute clinical symptoms of the antibody administration.

WHAT IS CLAIMED IS:

1. Use of an antibody which binds to a transferrin receptor (TfR) in the manufacture of a medicament for transporting a compound across the blood-brain barrier, wherein the antibody is coupled to the compound, and wherein the antibody binds TfR with low affinity and wherein 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 a subject such that reduction of red blood cell levels in the subject upon antibody administration is decreased or eliminated.

2. The use of claim 1, for treating a neurological disorder in the subject.

3. The use of claim 1 or claim 2, wherein the one or more properties are selected from the effector function of the antibody Fc region and the complement activation function of the antibody, and wherein the effector function or complement activation function has been reduced or eliminated relative to a wild-type antibody of the same isotype.

4. The use of claim 3, wherein the effector function is reduced or eliminated by a method selected from reduction of glycosylation of the antibody, modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function, and modification of the Fc region.

5. The use of claim 4, wherein the glycosylation of the antibody is reduced by a method selected from: production of the antibody in an environment that does not permit wild-type glycosylation; removal of carbohydrate groups already present on the antibody; and modification of the antibody such that wild-type glycosylation does not occur.

6. The use of claim 5, wherein the antibody is produced in a non-mammalian cell production system, or where the antibody is produced synthetically.

7. The use of any one of the preceding claims, wherein the Fc region of the antibody comprises a mutation at position 297 such that the wild-type asparagine residue at that position is replaced with another amino acid that interferes with glycosylation at that position.

8. The use of claim 4, wherein the effector function or complement activation function is reduced or eliminated by deletion of all or a portion of the Fc region, or by engineering the antibody such that it does not include an Fc region or non-Fc region competent for effector function or complement activation function, or wherein the modification is selected from: a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, and 439; a point mutation of the Fc region to impair binding to Clq selected from the following positions: 270, 322, 329, and 321, eliminating some or all of the Fc region, and a point mutation at position 132 of the CH1 domain

9. The use of any one of the preceding claims, wherein the antibody coupled to the compound is to be administered with a further compound selected from erythropoietin (EPO), an iron supplement, vitamin C, folic acid, and vitamin B 12, or wherein the further compound is red blood cells or reticulocytes from the same or another subject.

10. The use of any one of the preceding claims, wherein the subject is monitored for depletion of red blood cells.

11. The use of any one of the preceding claims, wherein the compound is a neurological disorder drug or an imaging agent.

12. The use of any one of the preceding claims, wherein the antibody does not impair the binding of the TfR to transferrin.

13. The use of any one of the preceding claims, wherein the antibody has an IC50 for the TfR from about 1 nM to about 100 μM, or from about 5 nM to about 100 μM, or from about 50 nM to about 100 μM, or from about 100 nM to about 100 μM.

14. The use of any one of the preceding claims, wherein the antibody has an affinity for the TfR from about 5 nM to about 50 μM.

15. The use of any one of the preceding claims, wherein the antibody coupled to the compound has an affinity for the TfR from about 30 nM to about 30 μM, or from about 30 nM to about 1 μM.

16. The use of any one of the preceding claims, wherein the antibody coupled to the compound has a dissociation half-life for the TfR from about 30 seconds to about 5 minutes, or from about 30 seconds to about 2 minutes.

17. The use of any one of the preceding claims, wherein the antibody is a multispecific antibody and the compound optionally forms one portion of the multispecific antibody.

18. The use of claim 2, wherein 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.

19. The use of any one of the preceding claims wherein the subject is human.

20. The use of claim 17 wherein the multispecific antibody comprises a first antigen binding site which binds the TfR and a second antigen binding site which binds a brain antigen.

21. The use of claim 20, wherein the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E4 (ApoE4), alphasynuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin I, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6.

22. The use of claim 20, wherein the multispecific antibody binds both TfR and BACE1, or wherein the multispecific antibody binds both TfR and Abeta.

23. A method of making an antibody useful for transporting a compound across the BBB with improved safety comprising selecting an antibody specific for a transferrin receptor (TfR)

that has low affinity for the TfR, and modifying one or more properties of the antibody to reduce the impact of the antibody on reticulocyte levels and/or reduce the severity or presence of acute clinical symptoms in a subject such that reduction of red blood cell levels in the subject upon antibody administration is decreased or eliminated compared to the unmodified antibody.

24. The method of claim 23, wherein the one or more properties is selected from the effector function of the antibody Fc region and the complement activation function of the antibody, and wherein the effector function or the complement activation function has been reduced or eliminated relative to a wild-type antibody of the same isotype.

25. The method of claim 24, wherein the effector function is reduced or eliminated by a method selected from reduction of glycosylation of the antibody, modification of the antibody isotype to an isotype that naturally has reduced or eliminated effector function, and modification of the Fc region.

26. The method of claim 25, wherein the glycosylation of the antibody is reduced by a method selected from: production of the antibody in an environment that does not permit wild-type glycosylation; removal of carbohydrate groups already present on the antibody; and modification of the antibody such that wild-type glycosylation does not occur.

27. The method of claim 26, wherein the antibody is produced in a non-mammalian cell production system, or where the antibody is produced synthetically.

28. The method of claim 26, wherein the Fc region of the antibody comprises a mutation at position 297 such that the wild-type asparagine residue at that position is replaced with another amino acid that interferes with glycosylation at that position.

29. The method of claim 24, wherein the effector function or the complement activation function is reduced or eliminated by deletion of all or a portion of the Fc region, or by engineering the antibody such that it does not include an Fc region competent for effector function or complement activation function, or the modification is selected from: a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, and 439; a point mutation of the Fc region to impair binding to Clq selected from the following positions: 270, 322, 329, and 321, eliminating some or all of the Fc region, and a point mutation at position 132 of the CH1 domain.

30. The method of any one of claims 23 to 29, wherein the antibody has an IC50 for the TfR from about 1 nM to about 100 μM, or from about 5 nM to about 100 μM, or from about 50 nM to about 100 μM, or from about 100 nM to about 100 μM.

31. The method of any one of claims 23 to 30, wherein the antibody is coupled with a therapeutic compound.

32. The method of any one of claims 23 to 30, wherein the antibody is a multispecific antibody which comprises a first antigen binding site which binds the TfR and a second antigen binding site which binds a brain antigen.

33. The method of any of claims 23 to 32, wherein the antibody has an affinity for the TfR from about 5 nM to about 50 μM.

34. The method of any of claims 23 to 33, wherein the antibody has a dissociation half-life for the TfR from about 30 seconds to about 5 minutes, or from about 30 seconds to about 2 minutes.

35. The method of claim 31, wherein the therapeutic compound is a neurological disorder drug.

36. The method of claim 32, wherein the brain antigen is selected from the group consisting of: beta-secretase 1 (BACEl), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6.

37. The method of claim 36, wherein (i) the multispecific antibody binds both TfR and BACEl, or (ii) the multispecific antibody binds both TfR and Abeta.

38. The method of any one of claims 23 to 37, wherein the antibody does not inhibit the binding of TfR to transferrin.

39. An antibody which binds to a TfR, wherein the affinity of the antibody for the TfR is from about 5 nM to about 50 μM or the dissociation half-life of the antibody for the TfR 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.

40. The antibody of claim 39, wherein the red blood cells are reticulocytes.

41. The antibody of claim 39 or claim 40, wherein at least one undesired side effect on red blood cells is selected from reduction of reticulocyte levels and acute clinical symptoms.

42. The antibody of any one of claims 39 to 41, wherein one or more properties is selected from the effector function of the antibody Fc region and the complement activation function of the antibody, and wherein the effector function or complement activation function has been reduced or eliminated relative to a wild-type antibody of the same isotype.

43. The antibody of claim 42, wherein the effector function is reduced or eliminated by a method selected from production of the antibody in an environment that does not permit wild-type glycosylation; removal of carbohydrate groups already present on the antibody; and modification of the antibody such that wild-type glycosylation does not occur.

44. The antibody of claim 43, wherein the antibody is produced in a non-mammalian cell production system, or where the antibody is produced synthetically.

45. The antibody of claim 43, wherein the Fc region of the antibody comprises a mutation at position 297 such that the wild-type asparagine residue at that position is replaced with another amino acid that interferes with glycosylation at that position.

46. The antibody of claim 42, wherein the effector function or complement activation function is reduced or eliminated by deletion of all or a portion of the Fc region, or by engineering the antibody such that it does not include an F c region competent for effector function, or wherein the modification is selected from: a point mutation of the Fc region to impair binding to one or more Fc receptors selected from the following positions: 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, 338, 340, 373, 376, 382, 388, 389, 414, 416, 419, 434, 435, 437, 438, and 439; a point mutation of the Fc region to impair binding to Clq selected from the following positions: 270, 322, 329, and 321; eliminating some or all of the Fc region, and a point mutation at position 132 of the CH1 domain.

47. The antibody of any one of claims 39 to 46, wherein: the antibody is coupled with a therapeutic compound.

48. The antibody of claim 47, wherein the therapeutic compound is a neurological disorder drug.

49. The antibody of claim 47, wherein the antibody is a multispecific antibody and the compound optionally forms one portion of the multispecific antibody.

50. The antibody of claim 49, wherein the antibody is a multispecific antibody which comprises a first antigen binding site which binds the TfR and a second antigen binding site which binds a brain antigen.

51. The antibody of claim 50, wherein the brain antigen is selected from the group consisting of: beta-secretase 1 (BACE 1), Abeta, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), tau, apolipoprotein E4 (ApoE4), alpha-synuclein, CD20, huntingtin, prion protein (PrP), leucine rich repeat kinase 2 (LRRK2), parkin, presenilin 1, presenilin 2, gamma secretase, death receptor 6 (DR6), amyloid precursor protein (APP), p75 neurotrophin receptor (p75NTR), and caspase 6.

52. The antibody of claim 51, wherein the multispecific antibody binds both TfR and BACEl, or wherein the multispecific antibody binds both TfR and Abeta.

53. The antibody of any one of claims 39 to 52, wherein the antibody does not inhibit the binding of TfR to transferrin.

54. Use of an antibody that binds with low affinity to a TfR and that does not impact red blood cell levels for the manufacture of a medicament for treating a neurological disorder.

55. Use of the antibody of any one of claims 39 to 53 for the manufacture of a medicament for treating a neurological disorder.

56. An antibody that binds with low affinity to a TfR and which does not impact red blood cell levels for use in treating a neurological disorder.

57. An antibody of any one of claims 39 to 53 for use in treating a neurological disorder.