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(54) **Titre : METHODES DE TRAITEMENT DE MALADIES NEUROLOGIQUES**
(54) **Title: METHODS OF TREATING NEUROLOGICAL DISEASES**

(57) **Abrégé/Abstract:**

Provided herein are methods of promoting removal of beta-amyloid (A β) plaque, methods of reducing neuroinflammation, and methods treating a neurological disorder (e.g., Alzheimer's Disease) with certain anti-CD22 antibodies or antigen-binding fragments. Exemplary antibodies, characteristics thereof, and methods of screening for additional therapeutic antibodies are also described herein.



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Abstract:

Provided herein are methods of promoting removal of beta-amyloid (A) plaque, methods of reducing neuroinflammation, and methods treating a neurological disorder (e.g., Alzheimer's Disease) with certain anti-CD22 antibodies or antigen-binding fragments. Exemplary antibodies, characteristics thereof, and methods of screening for additional therapeutic antibodies are also described herein.

METHODS OF TREATING NEUROLOGICAL DISEASES

[0001] This application claims priority to US Provisional Application No. 63/221,261, filed July 13, 2021, which is entirely incorporated herein by reference.

1. Field

[0002] The present invention relates to molecular biology and neurobiology, specifically, to the identification and uses of antibodies in the treatment of various neurological disorders relating to amyloid- β and/or neuroinflammation, such as Alzheimer's disease, amyloidoses and β -amyloid pathology.

2. Background

[0003] Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid- β (A β)-containing extracellular plaque. The causes leading to the conditions are generally unknown. It has been shown that removal of A β plaque can lead to clinical benefits in AD patients. However, there are only limited therapeutic options available for A β plaque removal, which are commonly associated with vascular side effects such as ARIA-E and ARIA-H. Accordingly, there are great needs for novel methods to remove A β plaques more efficiently and for therapeutic options for AD with less side effects. Methods provided in the present disclosure meet this need and provide related advantages.

3. Summary

[0004] Provided herein are method of promoting removal of beta-amyloid (A β) plaque in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.

[0005] Provided herein are also method of reducing neuroinflammation in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.

[0006] In some embodiments of the methods provided herein, the subject has clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy,

glaucoma, pre-eclampsia, cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessels.

[0007] Provided herein are also methods of treating an A β -related disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.

[0008] Provided herein are also methods of treating a disease or disorder associated with neuroinflammation in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.

[0009] In some embodiments, the A β -related or neuroinflammation-related disease or disorder is clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma, pre-eclampsia, cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessels. In some embodiments, the A β -related disease or disorder is Alzheimer's disease.

[0010] In some embodiments, the antibody or antigen-binding fragment used in the methods described herein is a monoclonal antibody or antigen-binding fragment.

[0011] In some embodiments, the antibody or antigen-binding fragment used in the methods disclosed herein is an antibody selected from the group consisting of an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, and an IgG4 antibody. In some embodiments, the antibody is an IgG1 antibody.

[0012] In some embodiments, the antibody or antigen-binding fragment used in the methods disclosed herein is selected from the group consisting of a Fab, a Fab', a F(ab')₂, a Fv, a scFv, a (scFv)₂, a single domain antibody (sdAb), and a heavy chain antibody (HCAb).

[0013] In some embodiments, the antibody or antigen-binding used in the methods disclosed herein is a chimeric antibody or antigen-binding, a humanized antibody or antigen-binding, or a human antibody or antigen-binding.

[0014] In some embodiments, the antibody or antigen-binding fragment used in methods disclosed herein specifically binds to CLLNFSCYGYPIQ (SEQ ID NO:11) and VFTRSELKFSPQWSHHGKIVTC (SEQ ID NO:12) of human CD22.

[0015] In some embodiments, the antibody or antigen-binding fragment used in methods disclosed herein comprises a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 that have the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively; and a heavy chain variable region (VH) comprising a VH CDR1, VH CDR2, and VH CDR3 that have the amino acid sequence of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.

[0016] In some embodiments, the antibody or antigen-binding fragment used in methods disclosed herein is a chimeric antibody or antigen-binding fragment. In some embodiments, the VL and VH of the chimeric antibody or antigen-binding fragment have the amino acid sequences of SEQ ID NO:7 and SEQ ID NO:8, respectively. In some embodiments, the chimeric antibody or antigen-binding fragment is SM03.

[0017] In some embodiments, the antibody or antigen-binding fragment used in methods disclosed herein is a human antibody or antigen-binding fragment. In some embodiments, the VL and VH of the humanized antibody or antigen-binding fragment have the amino acid sequences of SEQ ID NO:9 and SEQ ID NO:10, respectively. In some embodiments, the humanized antibody or antigen-binding fragment is SM06.

[0018] In some embodiments of the methods disclosed herein, the antibody or antigen-binding fragment is administered intravenously, intramuscularly, subcutaneously, intracranially, intrathecally, intraventricularly, intraperitoneally, intranasally, parenterally, topically, or intradermally. In some embodiments, the antibody or antigen-binding fragment is administered intravenously. In some embodiments, the antibody or antigen-binding fragment is administered subcutaneously.

[0019] In some embodiments of the methods disclosed herein, the antibody or antigen-binding fragment is administered in a therapeutically effective amount within the range of 1-50 mg/kg of body weight of the subject. In some embodiments, therapeutically effective amount is about 1, about 2, about 3, about 5, about 10, about 15, or about 30 mg/kg of body weight of the subject. In some embodiments, the antibody or antigen-binding fragment is administered in a therapeutically effective amount at 300 - 1,200 mg per dose. In some embodiments, the antibody or antigen-binding fragment is administered biweekly or monthly. In some embodiments, the antibody or antigen-binding fragment is administered in multiple doses. In some embodiments, the antibody or antigen-binding fragment is administered in multiple doses over a period of at least three months, at least six months, or at least one year.

[0020] In some embodiments of the methods disclosed herein, the antibody or antigen-binding fragment is administered in combination with a second therapeutic agent. In some embodiments, the

second therapeutic agent is an anti-beta-amyloid antibody, an anti-CD33 antibody, a Tau aggregation inhibitor, a Tau protein modulator, a cholinesterase inhibitor, an acetylcholinesterase inhibitor, an N-methyl D-aspartate (NMDA) antagonist, a β -secretase inhibitor, or an insulin sensitizer.

[0021] In some embodiments, the second therapeutic agent is an anti-beta-amyloid antibody selected from the group consisting of aducanumab, donanemab, gantenerumab, lecanemab, bapineuzumab, and solanezumab.

[0022] In some embodiments, the second therapeutic agent is an anti-CD33 antibody selected from the group consisting of AL003, gemtuzumab, lintuzumab, ozogamicin, vadastuximab talirine, and BI836858.

[0023] In some embodiments of the methods disclosed herein, the subject is a human subject.

[0024] Provided herein are also methods of inducing internalization of A β by a microglia cell, comprising contacting the microglia cell with an effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.

4. Brief Description of Drawings

[0025] **FIG.1** provides the Octet *in vitro* binding data showing the binding of A β to recombinant CD22 protein. As shown, A β bound to CD22 with a K_D of 6×10^{-8} M.

[0026] **FIG.2** provides the flow cytometry data showing the binding of A β to CD22-expressing cells. As shown, overexpression of human CD22 in HEK293 cells increased binding of FITC-A β onto the surface of HEK293 cells.

[0027] **FIG.3** provides immunocytochemistry PLA staining of HMC-3 cells after A β treatment. As shown, positive signal (arrows) indicates the physical interaction between A β and CD22 on cell surface of HMC-3 cells.

[0028] **FIG.4** provides immunofluorescence staining of CD22 in HMC3 cells. As shown, treatment of anti-CD22 antibody SM03 induced rapid internalization of CD22.

[0029] **FIG.5** provides the confocal data showing the internalization of an anti-CD22 antibody. As shown, FITC-SM03 was internalized within 30 mins after being added to HMC3 cells.

[0030] **FIG.6** provides the flow cytometry data for the rate of CD22 internalization induced by various anti-CD22 antibodies on HMC-3 cells. As shown, SM03 and SM06 induced the highest rate of internalization.

[0031] **FIG.7** provides the flow cytometry data showing the internalization of A β . As shown, treatment by SM03 and SM06 each enhanced phagocytosis of FITC-A β .

[0032] FIG.8 provides the data from luciferase assay measuring NFκB signaling. As shown, treatment of anti-CD22 antibody SM03 Fab reduced NFκB signaling in HMC-3 cells.

[0033] FIG.9 provides the data from ELISA measuring IL-6 secretion. As shown, treatment of anti-CD22 antibody SM03 Fab reduced IL-6 secretion by HMC-cells.

[0034] FIG.10 provides immunocytochemistry staining of 2,6-sialic acid *trans* binding on HMC-3 cells. As shown, SM03 and SM06 promoted *trans* binding of 2,6-sialic acid probe in HMC-3 cells.

5. Detailed Description

[0035] Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by amyloid-β (Aβ) containing extracellular plaque. Effective therapeutic options are still in great need. Disclosed herein are inventors' surprising discovery that toxic oligomeric Aβ1-42 binds to the CD22 expressed on human microglia, and that certain anti-CD22 antibodies can promote the removal of Aβ plaque by inducing internalization of CD22. Additionally, it was found that the anti-CD22 antibodies disclosed herein can also suppress autoimmunity in the central nervous system (CNS), providing further therapeutic benefits. Accordingly, in some embodiments, provided herein are methods of reducing Aβ accumulation and neuroinflammation, and treating related disease or disorder (*e.g.*, AD) via a CD22-mediated mechanism.

[0036] Dementia is estimated to increase from 50 million in 2010 to 113 million by 2050 worldwide. AD is the major cause of dementia and is often complicated with other neurodegenerative and cerebrospinal pathology. The neuropathologic features begin 15 to 20 years before obvious cognitive symptoms. Individuals with AD will progress from their normal baseline cognitive abilities through subtle changes of the preclinical stages, to obvious symptoms of brain dysfunction, termed prodromal AD, and finally, to AD dementia, which eventually impairs ability to perform activities of daily living (ADL) that they were previously independently performing, owing to cognitive deficits. The preclinical stage of AD is often considered the stage occurring before a clinical diagnosis of a cognitive disorder is considered. AD dementia is a synaptic dysfunction disease encompassing changes in molecular, cellular and connectome level. Clinical manifestation of AD patients includes dementia with amnesic or non-amnesic symptoms. This is often accompanied with verbal, visuospatial processing, and executive dysfunction. (Scharre, *Practical Neurology* (2019); Dubois *et al.*, *Alzheimers Dement* (2016), 12(3): 292–323.) Neuropathological hallmark of AD is characterized by excessive neuroinflammation, accumulation of extracellular Aβ containing plaque and hyper-phosphorylated tau protein. Aβ plaque is widespread in cortical and hippocampal region and causes

disrupted neuronal network via triggering neuronal loss and promotion of neuroinflammation via microglia.

[0037] A β is derived by amyloid precursor protein, which is a transmembrane protein enriched in neuronal surface membrane. Physiologically it is cleaved by α -secretase and later γ -secretase to form APP α . It functions in regulation of synaptic strength via modulating calcium flux and potassium channel. In amyloidogenic pathway, A β is cleaved by β -secretase and subsequently γ -secretase to form the A β 1-42. A β 1-42 misfolds in a β -sheet conformation and aggregates into toxic oligomeric form. In accordance with the amyloid cascade hypothesis, toxic oligomeric A β 1-42 aggregate into plaques which lead to neurotoxicity and dementia. Oligomeric A β 1-42 directly binds to metabotropic glutamate receptor 5 (mGluR5), N-Methyl-D-aspartic acid (NMDA) receptor, and other neuron receptors such as α -7 nicotinic acetylcholine receptor and insulin receptors to induce pathological changes in synaptic strength and morphology of dendritic spines. Oligomeric A β 1-42 could also stimulate microglia cell via pattern recognition receptors, namely Toll-like receptors (TLRs), Nod-like receptors (NLRs), RIG-like receptors (RLRs), AIM2-like receptors (ALRs), to trigger neuroinflammation. A β 1-42 induces pro-inflammatory cytokines, namely IL-1 β , IL-8 and TNF α , released by microglia. Also, it activates NLRP3 inflammasome cascade to secrete ASC specks protein (apoptosis-associated speck-like protein containing a caspase-1 recruitment domain) for the nucleation of further aggregation of A β 1-42.

[0038] Clearance of toxic oligomeric A β has been proposed to render clinical benefits in AD patients. As in 2021, of the 17 disease-modifying therapeutic in Phase III, 29% of them target A β as their mechanism of action (MOA). Anti-A β antibody, including aducanumab, donanemab, gantenerumab, lecanemab, bapineuzumab and solanezumab target various toxic form of A β via Fc receptor-mediated phagocytosis and non-Fc receptor-mediated clearance of A β .

[0039] Aducanumab is an IgG1 mAb selectively targeting soluble oligomer and insoluble fibrils of A β . Aducanumab binds amino acids 3-6 and recognizes conformational epitope on aggregated A β but not monomeric form. The binding of the antibody to A β aggregate triggers clearance by Fc gamma receptor-mediated phagocytosis, restoring calcium homeostasis of neuronal network in AD patients. Aducanumab was approved by FDA in June 2021 for the treatment of AD as the clinical studies consistently displayed reduction of amyloid plaques.

[0040] In addition to AD, other disease or disorders associated with abnormal accumulation of A β include, for example, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma, pre-eclampsia,

cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessels.

[0041] Before the present disclosure is further described, it is to be understood that the disclosure is not limited to the particular embodiments set forth herein, and it is also to be understood that the terminology used herein is for the purpose of describing particular embodiments, and is not intended to be limiting.

[0042] Unless otherwise defined herein, scientific and technical terms used in the present disclosures shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well-known and commonly used in the art.

[0043] The term “a” or “an” entity refers to one or more of that entity; for example, “a vector,” is understood to represent one or more vectors.

[0044] The term “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term “and/or” as used in a phrase such as “A and/or B” herein is intended to include “A and B,” “A or B,” “A” (alone), and B” (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0045] Ranges: throughout this disclosure, various aspects of the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 *etc.*, as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

[0046] Exemplary genes and polypeptides are described herein with reference to GenBank numbers, GI numbers and/or SEQ ID NOS. It is understood that one skilled in the art can readily identify homologous sequences by reference to sequence sources, including but not limited to GenBank (ncbi.nlm.nih.gov/genbank/) and EMBL (embl.org/).

5.1 A β clearance and neuroinflammation reduction

[0047] CD22 is a B cell restricted antigen that belongs to the immunoglobulin (Ig) superfamily. CD22 is a type I transmembrane sialoglycoprotein, also known as sialic acid binding immunoglobulin-type lectins (Siglecs). Human CD22 specifically binds to the structure: N-acetylneuraminic acid α (2-6) galactose (NeuAc- α (2-6)Gal), which is known to be expressed on hematopoietic cells, liver cells, lung epithelial cells, splenic cords and follicles, ileum stoma, heart stroma, blood vessels, skin epithelial cell secretions in eccrine sweat glands, colon stromal cells, liver sinusoids and stromal cells, and cells in central nervous system (CNS) such as the microglia cells in brain white matter (Gagneux *et al.*, *Journal of Biological Chemistry* (2003), 278(48):48245-50; Safaiyan *et al.*, *Neuron* (2021), 109(7):1100-17.e10). CD22 binds to its specific ligand distributed either on the same cell (*cis*-binding), or on a different cell (*trans*-binding). *Cis*-binding of CD22 is commonly found between neighboring molecules where the glycan binding site of a CD22 molecule is ligated to the glycan molecule of another CD22 or glycoprotein on the same cell, forming homo-oligomers or homo-multimers. *Cis*-binding of CD22 is demonstrated to exert a masking effect on the molecule, preventing the Siglec from forming cell to cell ligation (*trans*-binding). In resting B cells, CD22 is a prominent *cis*-ligand for itself, forming CD22 homo-oligomers. *Trans*-binding of CD22 results in the physical association of the Siglec with B-cell receptor (BCR) to exert a maximal inhibitory response, which is required for immune tolerance via eliciting inhibitory immunological signals (Pessutto *et al.*, 1987. *J. Immunol.* 138:98-103; Doody *et al.* 1995. *Science* 269:242-344; Lanoue *et al.* 2002. *Eur. J. Immunol.* 32:348-355; Courtney *et al.* 2009. *PNAS* 106:2500-2505). Additional information about human CD22, including its exemplary amino acid sequences can be found in public database such as UniProt (UniProt KB/Swiss-Prot ID: P20273) and GENBANK (NCBI Ref. NP_001172028.1, NP_001172029.1, NP_001172030.1, NP_001265346.1, NP_001762.2). An exemplary sequence is also provided below.

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1  MHLGPWLLL LVLEYLAFSD SSKWVFEHPE TLYAWEGACV WIPCTYRALD GDLESFILFH
61 NPEYNKNTSK FDGTRLYEST KDGKVPSEQK RVQFLGDKNK NCTLSIHPVH LNDSGQLGLR
121 MESKTEKWME RIHLNVSERP FPPHIQLPPE IQESQEVTLT CLLNFSCYGY PIQLQWLLEG
181 VPMRQAAVTS TSLTIKSVFT RSELKFSPQW SHHGKIVTCQ LQDADGKFLS NDTVQLNVKH
241 TPKLEIKVTP SDAIVREGDS VTMTCEVSSS NPEYTTVSWL KDGTSLLKKQN TFTLNLRVET
301 KDQSGKYCCQ VSNDVGPGRS EEVFLQVQYA PEPSTVQILH SPAVEGSQVE FLCMSLANPL
361 PTNYTWYHNG KEMQGRTEEK VHIPKILPWH AGTYSCVAEN ILGTGQRGPG AELDVQYPPK
421 KVTTVIQNPM PIREGDTVTL SCNYNSSNPS VTRYEWKPHG AWEEPSLGVL KIQNVGWDNT
481 TIACAACNSW CSWASPVALN VQYAPRDVRV RRIKPLSEIH SGNVSLQCD FSSSHPKEVQ
541 FFWEKNGRLL GKESQLNFDS ISPEDAGSYS CWNNSIGQT ASKAWTLEVL YAPRRLRVSM

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601 SPGDQVMEGK SATLTCESDA NPPVSHYTW F DWNNQSLPYH SQKLRLEPVK VQHSGAYWCQ
 661 GTNSVGKGRS PLSTLTVYYS PETIGRRVAV GLGSLAILI LAICGLKLQR RWKRTQSQQG
 721 LQENSSGQSF FVRNKKVRRR PLSEGP HSLG CYNPMMEDGI SYTTLRFP EM NIPRTGDAES
 781 SEMQRPPDC DDTVYTSALH KRQVGDYENV IPDFPEDEGI HYSELIQFGV GERPQAQENV
 841 DYVILKH (SEQ ID NO:11)

[0048] CD22 is highly expressed on microglia cells, which are the major antigen presenting cells in the CNS. The presented antigen (*e.g.*, A β or other self-antigen) can be recognized by infiltrating regulatory T cells which dampen the activation of microglia via secretion of IL-10. It was discovered by the inventors of present disclosure that certain anti-CD22 antibodies can induce internalization of CD22 in human microglia. It was also found that CD22 binds to oligomeric A β 1-42, and that the internalization of CD22 promotes clearance of A β , via both phagocytosis of CD22-bound A β 1-42 as well as macropinocytosis of soluble A β . Accordingly, in some embodiments, provided herein are methods of promoting removal of A β using anti-CD22 antibodies disclosed herein. In some embodiments, provided herein are methods of treating an A β -related disease or disorder using anti-CD22 antibodies disclosed herein. In some embodiments, the A β -related disease or disorder disease is AD.

[0049] As used herein and understood in the art, the “internalization” of CD22 refers to the endocytosis of CD22, which is the process by which a cell invaginates and engulfs CD22 molecules. There are three types of endocytotic process: pinocytosis, phagocytosis and receptor-mediated endocytosis. Pinocytosis is the process by which the cell engulfs small quantities of extracellular fluid (along with small particles that might be present) into the cell via the process of invagination, which is also referred to as “cell-drinking.” Phagocytosis is the process by which a relatively large molecule or organism (such as a bacterial cell) is engulfed by the cell. Receptor mediated endocytosis is the process by which the material binds directly onto the receptor protein of the cell-membrane, which initiates the process of invagination as well as the formation of a protein-covering layer known as the clathrin-coat. The vesicle that is formed in receptor mediated endocytosis contains this clathrin protein coating. CD22 on the cell membrane is internalized via the constitutive clathrin-mediated endocytosis. Once entered the endosomal compartments, the low pH environment frees up binding to its specific ligands (*e.g.*, CD22 binding in *cis*), and free CD22 returns to the cell surface for *cis*- or *trans*-ligand binding.

[0050] Current anti-A β antibody therapies for AD largely rely on Fc γ R-mediated phagocytosis to reduce amyloid burden, which are associated with the undesired side effects. Specifically, magnetic resonance imaging (MRI) abnormalities, such as signal changes representing “vasogenic edema” (VE)

and microhemorrhages (mH) have been observed in patients administered with amyloid-modifying therapeutic agents (anti-A β antibody) in a dose dependent manner. Amyloid-related imaging abnormalities (ARIA) is suggested by the Alzheimer's Association Research Roundtable Workgroup to describe vasogenic edema (ARIA-E) and microhemorrhages and hemosiderosis (ARIA-H). ARIA-E is largely caused by inflammation and tightly coupled to mAb that binds to fibril form of A β . The Fc γ R-mediated inflammation in microglia can take a major role. For example, the inflammation mediated by Fc γ R-induced proinflammatory cytokine releases exacerbate damage to vascular integrity. Crenzumab, a humanized IgG4 antibody targeting all form of A β is associated with less ARIA-E events, as the IgG4 Fc region has reduced association to Fc γ R of microglia as compared to, for example, an IgG1 Fc.

[0051] As such, anti-CD22 antibodies provided herein provide a novel mechanism of A β clearance that is not only highly efficient, but also associated with reduced vascular side effect like ARIA-E and/or ARIA-H. Unlike the A β -targeting antibodies, the anti-CD22 antibodies disclosed herein promotes A β clearance via CD22 internalization, which does not involve the cross-linking of the therapeutic antibodies with Fc γ R, thereby avoiding the subsequent pro-inflammatory response. Accordingly, provided here are methods of efficient A β clearance that have reduced vascular side effect. In some embodiments, methods provided herein do not cause vascular side effect.

[0052] The anti-CD22 antibodies (*e.g.*, SM03 or SM06) disclosed herein (a) promote *cis-trans* conversion of CD22 and/or (b) induce internalization of CD22. The anti-CD22 antibodies disclosed herein promote *cis-trans* conversion of CD22 by disrupting the *cis*-binding of CD22 on the surface of B-cells or microglia cells as well as accelerating the internalization of surface CD22. When the CD22 molecules are internalized with antibodies that disrupt the *cis*-binding of the homo-cluster, the CD22 molecules are recycled to the cell surface with the antibodies, where the antibodies sterically hinder further *cis*-binding, thereby promoting *trans*-binding of the recycled CD22. As such, the anti-CD22 antibodies disclosed herein can also promote immune-tolerance in both central and peripheral immune system by disrupting *cis*-binding of CD22 on B cell, B cell-derived cell lines and microglia, and promoting *cis-trans* conversion of 2,6-sialic acid binding of self-tissue, which allows regaining immune tolerance of self-tissue and dampens pro-inflammatory cytokine release. By promoting *trans*-binding of CD22 to neuronal 2,6-sialic acid, the anti-CD22 antibodies disclosed herein inhibit phagocytosis of synapse and neuronal death, as well as suppress NF- κ B signaling and interleukin-6 (IL-6) secretion in microglia cells. Thus, methods disclosed herein have the additional therapeutic benefit of reducing neuroinflammation. Accordingly, in some embodiments, provided herein are methods of reducing neuroinflammation using anti-CD22 antibodies disclosed herein. In some

embodiments, provided herein are methods of treating a neuroinflammation-associated disease or disorder using anti-CD22 antibodies disclosed herein.

[0053] Provided herein are methods of promoting removal of A β plaque in a subject in need thereof that comprise administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes *cis-trans* conversion of CD22 and/or (b) induces internalization of CD22. Provided herein are also methods of treating an A β -related disease or disorder in a subject in need thereof that comprise administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes *cis-trans* conversion of CD22 and/or (b) induces internalization of CD22. In some embodiments, the subject is a human. In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to human CD22. In some embodiments, the A β -related disease or disorder is AD. In some embodiments, the A β -related disease or disorder is preclinical AD.

[0054] In some embodiments, provided herein are uses of an antibody or antigen-binding fragment thereof that specifically binds to CD22 for removing A β plaque. In some embodiments, provided herein are uses of an antibody or antigen-binding fragment thereof that specifically binds to CD22 for the preparation of a medicament for the removal of A β plaque. In some embodiments, provided herein are uses of an antibody or antigen-binding fragment thereof that specifically binds to CD22 in the treatment of an A β -related disease or disorder. In some embodiments, provided herein are uses of an antibody or antigen-binding fragment thereof that specifically binds to CD22 for the preparation of a medicament for the treatment of an A β -related disease or disorder. In the uses described above, the antibody or antigen-binding fragment (a) promotes *cis-trans* conversion of CD22 and/or (b) induces internalization of CD22. In some embodiments, the A β -related disease or disorder is AD. In some embodiments, the A β -related disease or disorder is preclinical AD. In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to human CD22.

[0055] With the treatment of the CD22 antibodies disclosed herein, the rate of internalization of A β reached 5.86×10^{-6} pg/s/cell, as measured using ELISA of A β -treated microglia cell lysate (data not shown). In some embodiments of the uses or methods provided herein, A β is removed at a rate of 5.86×10^{-6} pg/s/cell.

[0056] Methods provided herein can treat an A β -related disease or disorder. The A β -related disease or disorder can be clinical or pre-clinical AD, prodromal AD, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid

angiopathy, glaucoma, pre-eclampsia, cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessel.

[0057] In some embodiments, methods provided herein can be used to treat AD. As described above and well known in the art, removal of oligomeric A β is known to provide clinical benefits in AD. The AD can be clinical AD, pre-clinical AD or prodromal AD. In some embodiments, methods provided herein can be used to treat clinical AD. In some embodiments, methods provided herein can be used to treat pre-clinical AD. In some embodiments, methods provided herein can be used to treat prodromal AD.

[0058] In some embodiments, methods provided herein can be used to treat Down's syndrome. In some embodiments, methods provided herein can be used to treat clinical or pre-clinical CAA. In some embodiments, methods provided herein can be used to treat Parkinson's disease. In some embodiments, methods provided herein can be used to treat multi-infarct dementia. In some embodiments, methods provided herein can be used to treat cerebral amyloid angiopathy. In some embodiments, methods provided herein can be used to treat glaucoma. In some embodiments, methods provided herein can be used to treat pre-eclampsia. In some embodiments, methods provided herein can be used to treat cognitive impairment. In some embodiments, methods provided herein can be used to treat memory loss. In some embodiments, methods provided herein can be used to treat a vascular disorder caused by pathogenic A β peptide in blood vessel.

[0059] Provided herein are also methods of reducing neuroinflammation in a subject in need thereof that comprise administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes *cis-trans* conversion of CD22 and/or (b) induces internalization of CD22. Provided herein are also methods of treating a disease or disorder associated with neuroinflammation in a subject in need thereof that comprise administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes *cis-trans* conversion of CD22 and/or (b) induces internalization of CD22. In some embodiments, the methods provided herein prevent synaptic phagocytosis and neuronal death. In some embodiments, the methods provided herein prevent synaptic phagocytosis and neuronal death by at least 20%, at least 30, at least 40%, at least over 50%, or at least over 60%. In some embodiments, the methods provided herein prevent synaptic phagocytosis and neuronal death by at least 50%. In some embodiments, the subject is a human. In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to human CD22.

[0060] In some embodiments, provided herein are uses of an antibody or antigen-binding fragment thereof that specifically binds to CD22 for reducing neuroinflammation. In some embodiments, provided herein are uses of an antibody or antigen-binding fragment thereof that specifically binds to CD22 for the preparation of a medicament for the reduction of neuroinflammation. In some embodiments, provided herein are uses of an antibody or antigen-binding fragment thereof that specifically binds to CD22 in the treatment of a disease or disorder associated with neuroinflammation. In some embodiments, provided herein are uses of an antibody or antigen-binding fragment thereof that specifically binds to CD22 for the preparation of a medicament for the treatment of a disease or disorder associated with neuroinflammation. In the uses described above, the antibody or antigen-binding fragment (a) promotes *cis-trans* conversion of CD22 and/or (b) induces internalization of CD22. In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to human CD22.

[0061] In some embodiments, the anti-CD22 antibody or antigen-binding fragment used in the methods described herein promotes *cis-trans* conversion of CD22. In some embodiments, the anti-CD22 antibody or antigen-binding fragment used in the methods described herein induces internalization of CD22. In some embodiments, the anti-CD22 antibody or antigen-binding fragment used in the methods described herein promotes *cis-trans* conversion of CD22 and induces internalization of CD22.

[0062] As used herein, the term “treat” and its grammatical equivalents in connection with a disease or a condition, or a subject having a disease or a condition refer to an action that suppresses, eliminates, reduces, and/or ameliorates a symptom, the severity of the symptom, and/or the frequency of the symptom associated with the disease or disorder being treated. For example, when used in reference to AD, the term “treat” and its grammatical equivalents refer to an action that reduces the severity of the disease, or retards or slows the progression of the disease, including, but not limited to (a) reducing the amount of A β plaque, or slowing the rate of accumulation of pathologic A β , or (b) delaying, ameliorating or minimizing one or more symptoms associated with AD, such as dementia or cognitive impairment.

[0063] As used herein, the term “administer” and its grammatical equivalents refer to the act of delivering, or causing to be delivered, a therapeutic or a pharmaceutical composition to the body of a subject by a method described herein or otherwise known in the art. The therapeutic can be a compound, a polypeptide, or a cell. Administering a therapeutic or a pharmaceutical composition includes prescribing a therapeutic or a pharmaceutical composition to be delivered into the body of a subject. Exemplary forms of administration include oral dosage forms, such as tablets, capsules,

syrups, suspensions; injectable dosage forms, such as intravenous (IV), intramuscular (IM), or intraperitoneal (IP); subcutaneous (SC), transdermal dosage forms, including creams, jellies, powders, or patches; buccal dosage forms; inhalation powders, sprays, suspensions, and rectal suppositories.

[0064] As used herein, the terms “effective amount,” “therapeutically effective amount,” and their grammatical equivalents refer to the administration of an agent to a subject, either alone or as a part of a pharmaceutical composition and either in a single dose or as part of a series of doses, in an amount that is capable of having any detectable, positive effect on any symptom, aspect, or characteristics of a disease, disorder or condition when administered to the subject. The therapeutically effective amount can be ascertained by measuring relevant physiological effects. The exact amount required vary from subject to subject, depending on the age, weight, and general condition of the subject, the severity of the condition being treated, the judgment of the clinician, and the like. A therapeutically effective amount is also one in which any toxic or detrimental effects of the therapeutic agent are outweighed by the therapeutically beneficial effects. An appropriate “effective amount” in any individual case can vary according to factors such as the disease state, age, sex, and weight of the individual, and can be determined by one of ordinary skill in the art using routine experimentation. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result, for example, the delay or prevention of the onset of a disease or disorder. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount is commonly less than the therapeutically effective amount.

[0065] The term “subject” as used herein refers to any animal (*e.g.*, a mammal), including, but not limited to, humans, non-human primates, canines, felines, rodents, and the like, which is to be the recipient of a particular treatment. A subject can be a human. A subject can be a patient with a particular disease.

5.2 CD22 Antibodies

[0066] As described in the section above, provided herein are methods and uses of antibodies that specifically bind to CD22, or antigen-binding fragment thereof. The term “antibody,” and its grammatical equivalents as used herein refer to an immunoglobulin molecule that recognizes and specifically binds a target, such as a protein, polypeptide, peptide, carbohydrate, polynucleotide, lipid, or a combination of any of the foregoing, through at least one antigen-binding site wherein the antigen-binding site is usually within the variable region of the immunoglobulin molecule. As used herein, the term encompasses intact polyclonal antibodies, intact monoclonal antibodies, single-domain antibodies (sdAbs; *e.g.*, camelid antibodies, alpaca antibodies), single-chain Fv (scFv)

antibodies, heavy chain antibodies (HCAbs), light chain antibodies (LCAbs), multispecific antibodies, bispecific antibodies, monospecific antibodies, monovalent antibodies, and any other modified immunoglobulin molecule comprising an antigen-binding site (*e.g.*, dual variable domain immunoglobulin molecules) as long as the antibodies exhibit the desired biological activity.

Antibodies also include, but are not limited to, mouse antibodies, rabbit antibodies, camel antibodies, primate antibodies, chimeric antibodies, humanized antibodies, and human antibodies. An antibody can be any of the five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, or subclasses (isotypes) thereof (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2), based on the identity of their heavy-chain constant domains referred to as alpha, delta, epsilon, gamma, and mu, respectively. In some embodiments, an antibody can comprise four polypeptide chains, two heavy (H) chains and two light (L) chains interconnected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region.

[0067] Unless expressly indicated otherwise, the term “antibody” as used herein include “antigen-binding fragment” of intact antibodies. The term “antigen-binding fragment” as used herein refers to a portion or fragment of an intact antibody that is the antigenic determining variable region of an intact antibody. Examples of antigen-binding fragments include, but are not limited to, Fab (a monovalent fragment consisting of the VL, VH, CL and CH1 domains without the hinge region), Fab' (a monovalent fragment consisting of the VL, VH, CL and CH1 domains attached with a hinge region), F(ab')₂ (a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region), Fd (a fragment consisting of the VH and CH1 domains), Fv (a fragment consisting of the VL and VH domains of a single arm of an antibody), linear antibodies, single chain antibody molecules (*e.g.*, scFv, which is a single polypeptide chain having VL and VH regions joined by recombinant means), heavy chain antibodies (HCAbs), light chain antibodies (LCAbs), disulfide-linked scFv (dsScFv), diabodies (bivalent, bispecific antibodies), tribodies, tetrabodies, minibodies, dual variable domain antibodies (DVD), single variable domain antibodies (sdAbs or dAbs; *e.g.*, camelid antibodies, alpaca antibodies), and single variable domain of heavy chain antibodies (VHH), and bispecific or multispecific antibodies formed from antibody fragments. A “bispecific” antibody is an artificial hybrid antibody having two different antigen binding sites, which recognize and specifically bind two different targets. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. *See, e.g.*, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148, 1547-1553 (1992).

[0068] An antibody or antigen-binding fragment thereof can be part of a larger immunoadhesion molecules, formed by covalent or noncovalent association of the antibody or antigen-binding fragment with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov *et al.* (1995) *Human antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antigen-binding fragments, such as Fab, Fab' and F(ab')₂ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antigen-binding fragments and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

[0069] The term "humanized antibody" as used herein refers to forms of non-human (*e.g.*, murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human sequences. Typically, humanized antibodies are human immunoglobulin. In some instances, the Fv framework region residues of a human immunoglobulin are replaced with the corresponding residues in an antibody from a non-human species. In some instances, residues of the CDRs are replaced by residues from the CDRs of a non-human species (*e.g.*, mouse, rat, hamster, camel) that have the desired specificity, affinity, and/or binding capability. The humanized antibody can be further modified by the substitution of additional residues either in the Fv framework region and/or within the replaced non-human residues to refine and optimize antibody specificity, affinity, and/or binding capability. The term "human antibody" as used herein refers to an antibody produced by a human or an antibody having an amino acid sequence corresponding to an antibody produced by a human made using any of the techniques known in the art.

[0070] The term "heavy chain" when used in reference to an antibody refers to a polypeptide chain of about 50-70 kDa, wherein the amino-terminal portion includes a variable region (VH) of about 120 to 130 or more amino acids and a carboxy-terminal portion that includes a constant region. In some embodiments, the heavy chain constant region is comprised of three domains, CH1, CH2 and CH3, and there is a short flexible hinge region connecting the CH1 and CH2 domains. The constant region can be one of five distinct types, referred to as alpha (α), delta (δ), epsilon (ϵ), gamma (γ) and mu (μ), based on the amino acid sequence of the heavy chain constant region. The distinct heavy chains differ in size: α , δ and γ contain approximately 450 amino acids, while μ and ϵ contain approximately 550 amino acids. When combined with a light chain, these distinct types of heavy chains give rise to five well known classes of antibodies, IgA, IgD, IgE, IgG and IgM, respectively,

including four subclasses of IgG, namely IgG1, IgG2, IgG3 and IgG4. A heavy chain can be a human heavy chain.

[0071] The term “light chain” when used in reference to an antibody refers to a polypeptide chain of about 25 kDa, wherein the amino-terminal portion includes a variable region of about 100 to about 110 or more amino acids and a carboxy-terminal portion that includes a constant region. The light chain constant region is comprised of one domain, CL. The approximate length of a light chain is 211 to 217 amino acids. There are two distinct types, referred to as kappa (κ) or lambda (λ) based on the amino acid sequence of the constant domains. Light chain amino acid sequences are well known in the art. A light chain can be a human light chain.

[0072] The term “variable domain” or “variable region” refers to a portion of the light or heavy chains of an antibody that is generally located at the amino-terminal of the light or heavy chain and has a length of about 120 to 130 amino acids in the heavy chain and about 100 to 110 amino acids in the light chain, and are used in the binding and specificity of each particular antibody for its particular antigen. The variable domains differ extensively in sequence between different antibodies. The variability in sequence is concentrated in the CDRs while the less variable portions in the variable domain are referred to as framework regions (FR). The CDRs of the light and heavy chains are primarily responsible for the interaction of the antibody with antigen. In some embodiments, each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Numbering of amino acid positions used herein is according to the EU Index, as in Kabat *et al.* (1991) Sequences of proteins of immunological interest. (U.S. Department of Health and Human Services, Washington, D.C.) 5th ed.

[0073] A CDR refers to one of three hypervariable regions (H1, H2 or H3) within the non-framework region of the immunoglobulin (Ig or antibody) VH β -sheet framework, or one of three hypervariable regions (L1, L2 or L3) within the non-framework region of the antibody VL β -sheet framework. Accordingly, CDRs are variable region sequences interspersed within the framework region sequences. CDR regions are well known to those skilled in the art and have been defined by a variety of methods/systems. These systems and/or definitions have been developed and refined over years and include Kabat, Chothia, IMGT, AbM, and Contact. For example, Kabat defines the regions of most hypervariability within the antibody variable (V) domains (Kabat *et al.*, *J. Biol. Chem.* 252:6609-6616 (1977); Kabat, *Adv. Prot. Chem.* 32: 1-75 (1978)). The Chothia definition is based on the location of the structural loop regions, which defines CDR region sequences as those residues that are not part of the conserved β -sheet framework, and thus are able to adapt different

conformations (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). Both terminologies are well recognized in the art. Additionally, the IMGT system is based on sequence variability and location within the structure of the variable regions. The AbM definition is a compromise between Kabat and Chothia. The Contact definition is based on analyses of the available antibody crystal structures. Software programs (*e.g.*, abYsis) are available and known to those of skill in the art for analysis of antibody sequence and determination of CDRs. The positions of CDRs within a canonical antibody variable domain have been determined by comparison of numerous structures (Al-Lazikani *et al.*, *J. Mol. Biol.* 273:927-948 (1997); Morea *et al.*, *Methods* 20:267-279 (2000)). Because the number of residues within a hypervariable region varies in different antibodies, additional residues relative to the canonical positions are conventionally numbered with a, b, c and so forth next to the residue number in the canonical variable domain numbering scheme (Al-Lazikani *et al.*, *supra* (1997)). Such nomenclature is similarly well known to those skilled in the art.

[0074] For example, CDRs defined according to either the Kabat (hypervariable) or Chothia (structural) designations, are set forth in the table below.

	Kabat ¹	Chothia ²	Loop Location
VHCDRI	31-35	26-32	linking B and C strands
VHCDR2	50-65	53-55	linking C' and C'' strands
VHCDR3	95-102	96-101	linking F and G strands
VLCDRI	24-34	26-32	linking B and C strands
VLCDR2	50-56	50-52	linking C' and C'' strands
VLCDR3	89-97	91-96	linking F and G strands

¹Residue numbering follows the nomenclature of Kabat *et al.*, *supra*

²Residue numbering follows the nomenclature of Chothia *et al.*, *supra*

[0075] One or more CDRs also can be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin. An immunoadhesin can incorporate the CDR(s) as part of a larger polypeptide chain, can covalently link the CDR(s) to another polypeptide chain, or can incorporate the CDR(s) noncovalently. The CDRs permit the immunoadhesin to bind to a particular antigen of interest.

[0076] The terms “epitope” and “antigenic determinant” are used interchangeably herein and refer to the site on the surface of a target molecule to which an antibody or antigen-binding fragment binds, such as a localized region on the surface of an antigen. The target molecule can comprise, a protein, a peptide, a nucleic acid, a carbohydrate, or a lipid. An epitope having immunogenic activity is a

portion of a target molecule that elicits an immune response in an animal. An epitope of a target molecule having antigenic activity is a portion of the target molecule to which an antibody binds, as determined by any method well known in the art, including, for example, by an immunoassay. Antigenic epitopes need not necessarily be immunogenic. Epitopes often consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics. The term, “epitope” includes linear epitopes and conformational epitopes. A region of a target molecule (*e.g.*, a polypeptide) contributing to an epitope can be contiguous amino acids of the polypeptide or the epitope can come together from two or more non-contiguous regions of the target molecule. The epitope may or may not be a three-dimensional surface feature of the target molecule. Epitopes formed from contiguous amino acids (also referred to as linear epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding (also referred to as conformational epitopes) are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5, 6, 7, or 8-10 amino acids in a unique spatial conformation.

[0077] The term “specifically binds,” as used herein, means that a polypeptide or molecule interacts more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to the epitope, protein, or target molecule than with alternative substances, including related and unrelated proteins. A binding moiety (*e.g.*, antibody) that specifically binds a target molecule (*e.g.*, antigen) can be identified, for example, by immunoassays, ELISAs, Bio-Layer Interferometry (“BLI”), SPR (*e.g.*, Biacore), or other techniques known to those of skill in the art. Typically, a specific reaction will be at least twice background signal or noise and can be more than 10 times background. *See, e.g.*, Paul, ed., 1989, FUNDAMENTAL IMMUNOLOGY SECOND EDITION, Raven Press, New York at pages 332-336 for a discussion regarding antibody specificity. A binding moiety that specifically binds a target molecule can bind the target molecule at a higher affinity than its affinity for a different molecule. In some embodiments, a binding moiety that specifically binds a target molecule can bind the target molecule with an affinity that is at least 20 times greater, at least 30 times greater, at least 40 times greater, at least 50 times greater, at least 60 times greater, at least 70 times greater, at least 80 times greater, at least 90 times greater, or at least 100 times greater, than its affinity for a different molecule. In some embodiments, a binding moiety that specifically binds a particular target molecule binds a different molecule at such a low affinity that binding cannot be detected using an assay described herein or otherwise known in the art. In some embodiments, “specifically binds” means, for instance, that a binding moiety binds a molecule target with a K_D of about 0.1 mM or less. In some embodiments, “specifically binds” means that a polypeptide or

molecule binds a target with a K_D of at about 10 μM or less or about 1 μM or less. In some embodiments, “specifically binds” means that a polypeptide or molecule binds a target with a K_D of at about 0.1 μM or less, about 0.01 μM or less, or about 1 nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include a polypeptide or molecule that recognizes a protein or target in more than one species. Likewise, because of homology within certain regions of polypeptide sequences of different proteins, specific binding can include a polypeptide or molecule that recognizes more than one protein or target. It is understood that, in some embodiments, a binding moiety (*e.g.*, antibody) that specifically binds a first target may or may not specifically bind a second target. As such, “specific binding” does not necessarily require (although it can include) exclusive binding, *i.e.*, binding to a single target. Thus, a binding moiety (*e.g.*, antibody) can, in some embodiments, specifically bind more than one target. For example, an antibody can, in certain instances, comprise two identical antigen-binding sites, each of which specifically binds the same epitope on two or more proteins. In certain alternative embodiments, an antibody can be bispecific and comprise at least two antigen-binding sites with differing specificities.

[0078] The term “binding affinity” as used herein generally refers to the strength of the sum total of noncovalent interactions between a binding moiety and a target molecule (*e.g.*, antigen). The binding of a binding moiety and a target molecule is a reversible process, and the affinity of the binding is typically reported as an equilibrium dissociation constant (K_D). K_D is the ratio of a dissociation rate (k_{off} or k_d) to the association rate (k_{on} or k_a). The lower the K_D of a binding pair, the higher the affinity. K_A is the equilibrium association constant, which is also the reciprocal of the equilibrium dissociation constant, *i.e.*, $= 1 / K_D$. For an antibody-antigen interaction, K_D can be calculated as the ratio of the products of concentrations of free antibody and free antigen over the concentrations of antibody-antigen complex, *i.e.*, $[\text{antigen}] \times [\text{antibody}] / [\text{antigen-antibody}]$.

[0079] A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present disclosure. Specific illustrative embodiments include the following. In some embodiments, the “ K_D ” or “ K_D value” can be measured by assays known in the art, for example by a binding assay. The K_D may be measured in a radiolabeled antigen binding assay (RIA) (Chen, *et al.*, (1999) *J. Mol Biol* 293:865-881). The K_D or K_D value can also be measured by using biolayer interferometry (BLI) using, for example, the Gator system (Probe Life), or the Octet-96 system (Sartorius AG). The K_D or K_D value can also be measured by using surface plasmon resonance assays by using a BIAcore system (*e.g.*, Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ).

[0080] The term “variant” as used herein in relation to a protein or a polypeptide with particular sequence features (the “reference protein” or “reference polypeptide”) refers to a different protein or polypeptide having one or more (such as, for example, about 1 to about 25, about 1 to about 20, about 1 to about 15, about 1 to about 10, or about 1 to about 5) amino acid substitutions, deletions, and/or additions as compared to the reference protein or reference polypeptide. The changes to an amino acid sequence can be amino acid substitutions. The changes to an amino acid sequence can be conservative amino acid substitutions. A functional fragment or a functional variant of a protein or polypeptide maintains the basic structural and functional properties of the reference protein or polypeptide.

[0081] The terms “polypeptide,” “peptide,” “protein,” and their grammatical equivalents as used interchangeably herein refer to polymers of amino acids of any length, which can be linear or branched. It can include unnatural or modified amino acids or be interrupted by non-amino acids. A polypeptide, peptide, or protein can also be modified with, for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification.

[0082] The terms “polynucleotide,” “nucleic acid,” and their grammatical equivalents as used interchangeably herein mean polymers of nucleotides of any length and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase. A nucleic acid molecule can be single-stranded or double-stranded.

[0083] As used herein, the term “encode” and its grammatical equivalents refer to the inherent property of specific sequences of nucleotides in a polynucleotide or a nucleic acid, such as a gene, a cDNA, or an mRNA, to serve as templates for synthesis of other polymers and macromolecules in biological processes having either a defined sequence of nucleotides (*i.e.*, rRNA, tRNA and mRNA) or a defined sequence of amino acids and the biological properties resulting therefrom. Thus, a gene encodes a protein if transcription and translation of mRNA corresponding to that gene produces the protein. Unless otherwise specified, a “nucleotide sequence encoding an amino acid sequence” includes all nucleotide sequences that are degenerate versions of each other and that encode the same amino acid sequence. Nucleotide sequences that encode proteins and RNA can include introns.

[0084] A polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is “isolated” is a polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. Isolated polypeptides, peptides, proteins, antibodies, polynucleotides, vectors, cells, or compositions include those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a

polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure. In some embodiments, a polypeptide, peptide, protein, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially free of other cellular material and/or chemicals.

[0085] The terms “identical,” percent “identity,” and their grammatical equivalents as used herein in the context of two or more polynucleotides or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that can be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two polynucleotides or polypeptides provided herein are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the amino acid sequences that is at least about 10 residues, at least about 20 residues, at least about 40-60 residues, at least about 60-80 residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as the coding region of a target protein or an antibody. In some embodiments, identity exists over a region of the nucleotide sequences that is at least about 10 bases, at least about 20 bases, at least about 40-60 bases, at least about 60-80 bases in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 bases, such as at least about 80-1000 bases or more, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, such as a nucleotide sequence encoding a protein of interest.

[0086] A “conservative amino acid substitution” as used herein, is one in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Amino acid or residue that is “conservatively similar” as used herein refers to non-identical amino acid residue having similar side chains. Families of amino acid residues having similar side chains have been

defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[0087] The term “vector,” and its grammatical equivalents as used herein refer to a vehicle that is used to carry genetic material (*e.g.*, a polynucleotide sequence), which can be introduced into a host cell, where it can be replicated and/or expressed. Vectors applicable for use include, for example, expression vectors, plasmids, phage vectors, viral vectors, episomes and artificial chromosomes, which can include selection sequences or markers operable for stable integration into a host cell’s chromosome. Additionally, the vectors can include one or more selectable marker genes and appropriate expression control sequences. Selectable marker genes that can be included, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like which are well known in the art. When two or more polynucleotides are to be co-expressed, both polynucleotides can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding polynucleotides can be operationally linked to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The introduction of polynucleotides into a host cell can be confirmed using methods well known in the art. It is understood by those skilled in the art that the polynucleotides are expressed in a sufficient amount to produce a desired product (*e.g.*, an anti-CD22 antibody or antigen-binding fragment), and it is further understood that expression levels can be optimized to obtain sufficient expression using methods well known in the art.

[0088] As used herein, the term “host cell” refers to a cell into which a genetical material, such as a recombinant expression vector can be introduced or has been introduced. Host cells include not only the subject cell introduced with the exogenous genetic material, but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not be identical to the parent cell.

[0089] As used herein and understood in the art, “EC” means effective concentration of an agent (*e.g.*, antibody), and is commonly used in dose-response curves. The “effect” of the agent can be a positive (activatory) effect or a negative effect. The term “EC50” refers to the concentration of an active agent (*e.g.*, antibody) that gives half-maximal response. Also as used herein and understood in

the art, “IC” means concentration of an agent that has an inhibitory effect, and is also commonly used for dose-response curves. The term “IC₅₀” refers to the concentration of an agent (*e.g.*, antibody) where the activity that it inhibits is reduced by half.

5.2.1 Exemplary CD22 Antibodies

[0090] As described in the section above, provided herein are methods and uses of antibodies that specifically bind to CD22, or antigen-binding fragment thereof that (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22. The antibodies and antigen-binding fragment provided herein can be used in A β removal, reduction of neuroinflammation, or both. The antibodies and antigen-binding fragment provided herein can also be used in the treatment of a disease or disorder that is associated with A β and/or neuroinflammation. In some embodiments, the antibodies used in methods provided herein specifically bind human CD22.

[0091] In some embodiments, the anti-CD22 antibody that can be used in methods disclosed herein is an IgA, IgD, IgE, IgG, or IgM antibody. In some embodiments, the antibody is an IgA antibody. In some embodiments, the antibody is an IgD antibody. In some embodiments, the antibody is an IgE antibody. In some embodiments, the antibody is an IgG antibody. In some embodiments, the antibody is an IgM antibody. In some embodiments, the antibodies provided herein can be an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG2 antibody. In some embodiments, the antibody is an IgG3 antibody. In some embodiments, the antibody is an IgG4 antibody. In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region or any of the above constant region with the glycosylation site and/or the glycoforms at the glycosylation site modified.

[0092] In some embodiments, used in methods disclosed herein are antigen-binding fragments of an anti-CD22 antibody. In some embodiments, antigen-binding fragments provided herein can be a single domain antibody (sdAb), a heavy chain antibody (HCAb), a Fab, a Fab', a F(ab')₂, a Fv, a single-chain variable fragment (scFv), or a (scFv)₂. In some embodiments, the antigen-binding fragment of an anti-CD22 antibody is a single domain antibody (sdAb). In some embodiments, the antigen-binding fragment of an anti-CD22 antibody is a heavy chain antibody (HCAb). In some embodiments, the antigen-binding fragment of an anti-CD22 antibody is a Fab. In some embodiments, the antigen-binding fragment of an anti-CD22 antibody is a Fab'. In some embodiments, the antigen-binding fragment of an anti-CD22 antibody is a F(ab')₂. In some embodiments, the antigen-binding fragment of an anti-CD22 antibody is a Fv. In some embodiments, the antigen-binding fragment of an anti-CD22 antibody is a scFv. In some embodiments, the antigen-

binding fragment of an anti-CD22 antibody is a disulfide-linked scFv [(scFv)₂]. In some embodiments, the antigen-binding fragment of an anti-CD22 antibody is a diabody (dAb).

[0093] In some embodiments, used in methods disclosed herein are recombinant anti-CD22 antibodies or antigen-binding fragments. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein comprise monoclonal antibodies or antigen-binding fragments. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein comprise polyclonal antibodies or antigen-binding fragments. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein comprise camelid (*e.g.*, camels, dromedary and llamas) antibodies or antigen-binding fragments. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein comprise chimeric antibodies or antigen-binding fragments. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein comprise humanized antibodies or antigen-binding fragments. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein comprise human antibodies or antigen-binding fragments. In some embodiments, provided herein are anti-CD22 humanized scFvs. In some embodiments, provided herein are anti-CD22 human humanized Fabs.

[0094] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods provided herein are isolated. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods provided herein are substantially pure.

[0095] In some embodiments, the anti-CD22 antibody or antigen-binding fragment used in methods provided herein comprises a monovalent antigen-binding site. In some embodiments, an anti-CD22 antibody or antigen-binding fragment comprises a monospecific binding site. In some embodiments, an anti-CD22 antibody or antigen-binding fragment comprises a bivalent binding site.

[0096] In some embodiments, a monoclonal antibody is modified by using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light chain and heavy chain of a mouse monoclonal antibody are replaced with the constant regions of a human antibody to generate a chimeric antibody. In some embodiments, the constant regions are truncated or removed to generate a desired antibody fragment of a monoclonal antibody. In some embodiments, site-directed or high-density mutagenesis of the variable region(s) is used to optimize specificity and/or affinity of a monoclonal antibody.

[0097] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in methods disclosed herein is a humanized antibody or antigen-binding fragment. Various methods for generating humanized antibodies are known in the art. Methods are known in the art for achieving high affinity binding with humanized antibodies. A non-limiting example of such a method is

hypermutation of the variable region and selection of the cells expressing such high affinity antibodies (affinity maturation). In addition to the use of display libraries, the specified antigen (*e.g.*, recombinant CD22 or an epitope thereof) can be used to immunize a non-human animal, *e.g.*, a rodent. In certain embodiments, rodent antigen-binding fragments (*e.g.*, mouse antigen-binding fragments) can be generated and isolated using methods known in the art and/or disclosed herein. In some embodiments, a mouse can be immunized with an antigen (*e.g.*, recombinant CD22 or an epitope thereof).

[0098] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in methods disclosed herein is a human antibody or antigen-binding fragment. Human antibodies can be prepared using various techniques known in the art. In some embodiments, human antibodies are generated from immortalized human B lymphocytes immunized *in vitro*. In some embodiments, human antibodies are generated from lymphocytes isolated from an immunized individual. In any case, cells that produce an antibody directed against a target antigen can be generated and isolated. In some embodiments, a human antibody is selected from a phage library, where that phage library expresses human antibodies. Alternatively, phage display technology can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable region gene repertoires from unimmunized donors. Techniques for the generation and use of antibody phage libraries are well-known in the art. Once antibodies are identified, affinity maturation strategies known in the art, including but not limited to, chain shuffling and site-directed mutagenesis, can be employed to generate higher affinity human antibodies. In some embodiments, human antibodies are produced in transgenic mice that contain human immunoglobulin loci. Upon immunization these mice are capable of producing the full repertoire of human antibodies in the absence of endogenous immunoglobulin production.

[0099] The specific CDR sequences defined herein are generally based on a combination of Kabat and Chothia definitions. However, it is understood that reference to a heavy chain CDR or CDRs and/or a light chain CDR or CDRs of a specific antibody encompass all CDR definitions as known to those of skill in the art. Anti-CD22 antibodies or antigen-binding fragments that can be used in methods provided herein include SM03 and SM06, and their variations and derivations.

[00100] The term “SM03” refers to a chimeric antibody against human CD22 (hCD22). Sequence features of SM03 are provided in the table below. Additional description of the structural and functional features of SM03 can be found in, *e.g.*, Yang *et al.* (2006), *Chinese J New Drug* 15(3):186-92; and Chinese Pat. No. ZL03123054.7, incorporated herein in their entirety by reference.

[00101] The term “SM06” refers to a framework-patched or humanized version of chimeric antibody SM03 reengineered to reduce its potential immunogenicity and exhibiting affinity and specificity against hCD22. Sequence features of SM06 are provided in the table below. Additional description of the structural and functional features of SM06 can be found in, *e.g.*, Liang *et al.* (2006) *Chinese J New Drug* 15(21):1832-36; Chinese Patent No. ZL 01144894.6, and US Pat. No. 7,321,026 B2 & 7,338,659 B2, incorporated herein in their entirety by reference.

Table 1: CDR sequences of SM03 and SM06

Antibody	VL CDR1	VL CDR2	VL CDR3
SM03/SM06	RASQDISNYLN (SEQ ID NO:1)	YTSILHS (SEQ ID NO:2)	QQGNTLPWT (SEQ ID NO:3)
	VH CDR1	VH CDR2	VH CDR3
	IYDMS (SEQ ID NO:4)	YISSGGGTTYYPDTVKG (SEQ ID NO:5)	HSGYGSSYGVLFAFAY (SEQ ID NO:6)

Table 2: Variable regions of SM03 and SM06

Antibody	VL	VH
SM03	DIQLTQTSSLSASLGDRVTISCRAS QDISNYLNWYQQKPDGTVKLLIYYT SILHSGVPSRFSGSGSGTDYSLTISNL EQEDFATYFCQQGNTLPWTFGGGT KLEIKR (SEQ ID NO:7)	QVQLQESGGGLVKPGGSLKLSCAAS GFAFSIYDMSWVRQTPEKRLEWVA YISSGGGTTYYPDTVKGRTISRDN KNTLYLQMSSLKSEDTAMYYCARH SGYGSSYGVLFAFAYWGQGILVT VSS (SEQ ID NO:8)
SM06	DIQLTQSPSSLSASVGDRVTISCRAS QDISNYLNWYQQKPGKAPKLLIYYT SILHSGVPSRFSGSGSGTEFTLTISL QPEDFATYFCQQGNTLPWTFGGGT KVEIKR (SEQ ID NO:9)	EVQLVESGGGLVQPGGSLRLSCAAS GFSFSIYDMSWVRQAPGKGLEWVA YISSGGGTTYYPDTVKGRTISRDN KNSLYLQMNSLRVEDTALYYCARH SGYGSSYGVLFAFAYWGQGILVT VSS (SEQ ID NO:10)

[00102] In some embodiments, anti-CD22 antibodies or antigen-binding fragments that can be used in methods provided herein comprise one, two, three, four, five, and/or six CDRs of SM03/SM06. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments comprise a VL comprising one, two, and/or three, VL CDRs from Table 1. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein comprise a VH comprising one, two, and/or three VH CDRs from Table 1. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein comprise one, two, and/or three VL CDRs and one, two, and/or three VH CDRs from Table 1.

[00103] In some embodiments, the antibodies or antigen-binding fragments thereof that can be used in methods provided herein comprise a light chain variable region (VL) comprising (1) a light chain CDR1 (VL CDR1) having the amino acid sequence of SEQ ID NO:1; (2) a light chain CDR2 (VL CDR2) having the amino acid sequence of SEQ ID NO:2; or (3) a light chain CDR3 (VL CDR3) having the amino acid sequence of SEQ ID NO:3; or a variant thereof having up to about 3, about 5, about 8, about 10, about 12, or about 15 amino acid substitutions, additions, and/or deletions in the VL CDRs. In some embodiments, the variant has about 5 amino acid substitutions, additions, and/or deletions in the VL CDRs. In some embodiments, the antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprise a VL comprising (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:1; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:2; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:3; or a variant thereof having up to about 3, about 5, about 8, about 10, about 12, or about 15 amino acid substitutions, additions, and/or deletions in the VL CDRs. In some embodiments, the variant has up to about 5 amino acid substitutions, additions, and/or deletions in the VL CDRs.

[00104] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a heavy chain variable region (VH) comprising (1) a heavy chain CDR1 (VH CDR1) having the amino acid sequence of SEQ ID NO:4; (2) a heavy chain CDR2 (VH CDR2) having the amino acid sequence of SEQ ID NO:5; or (3) a heavy chain CDR3 (VH CDR3) having the amino acid sequence of SEQ ID NO:6; or a variant thereof having up to about 3, about 5, about 8, about 10, about 12, or about 15 amino acid substitutions, additions, and/or deletions in the VH CDRs. In some embodiments, the variant has up about 5 amino acid substitutions, additions, and/or deletions in the VH CDRs. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VH comprising (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:4; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:6; or a variant thereof having up to about 3, about 5, about 8, about 10, about 12, or about 15 amino acid substitutions, additions, and/or deletions in the VH CDRs. In some embodiments, the variant has up about 5 amino acid substitutions, additions, and/or deletions in the VH CDRs.

[00105] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22, comprising (a) a VL comprising (1) a VL CDR1 having the amino acid sequence of SEQ ID NO:1; (2) a VL CDR2 having the amino acid sequence of SEQ ID NO:2; and (3) a VL CDR3 having the amino acid sequence of SEQ ID NO:3; or a variant thereof having up to about 5 amino acid substitutions, additions, and/or deletions in the VL CDRs; and (b) a VH

comprising (1) a VH CDR1 having the amino acid sequence of SEQ ID NO:4; (2) a VH CDR2 having the amino acid sequence of SEQ ID NO:5; and (3) a VH CDR3 having the amino acid sequence of SEQ ID NO:6; or a variant thereof having up to about 5 amino acid substitutions, additions, and/or deletions in the VH CDRs.

[00106] It is well known in the art that VH CDR3 and VL CDR3 domains play an important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, in some embodiments, the anti-CD22 antibodies or antigen-binding fragments thereof that can be used in methods disclosed herein can have the appropriate association/dissociation kinetics with human CD22 and have the VH CDR3 and VL CDR3 that are structurally identical to or related to those of SM03 and/or SM06. A consensus motif for the SM03 VL CDR3 comprising the amino acid sequence: Q-Q-G-N-T-L-P-W-T (SEQ ID NO:3) can be modified by substituting one or more of the amino acid(s) to adjust the antibody affinity without changing its binding specificity, or alternatively be replaced by the VL CDR3 of an irrelevant human antibody that exhibits sufficient similarities to the SM03 VL CDR3 using criteria as described in Chinese Pat. No. ZL200880024788.2, which is incorporated herewith by reference. Similarly, a consensus motif for the SM03 VH CDR3 comprising the amino acid sequence: H-S-G-Y-G-S-S-Y-G-V-L-F-A-Y (SEQ ID NO:6) can be modified by substituting one or more of the amino acid(s) to adjust the antibody affinity without changing its binding specificity, or alternatively be replaced by the VH CDR3 of an irrelevant human antibody that exhibits sufficient similarities to the SM03 VH CDR3 using criteria as described in Chinese Pat. No.

ZL200880024788.2, which is incorporated herewith by reference. The skilled artisan would appreciate that certain substitution(s) of amino acids within the CDR3 domains would not change the epitope specificity of the antibody, in particular substitutions with conservative amino acids. As such, in some embodiments, the CDR3 of the antibodies or antigen-binding fragments provided herein (*e.g.*, SM03 or SM06) can be replaced with the CDR3 from a human or primate antibody that (1) is identical in the number of residues and exhibits 50% or higher sequence homology to the SM03 CDR3, (2) contains at least one, preferably more, aromatic residue(s) that is (are) identical or conservatively similar to the residue(s) at corresponding position(s) in the SM03 CDR3, (3) contains at least one, preferably more, charged residue(s) that is (are) identical or conservatively similar to the residue(s) at corresponding position(s) in the SM03 CDR3, and/or (4) contains at least one, preferably more, amino acid residue(s) that is/are identical or conservatively similar to the residue(s) at corresponding position(s) in the SM03 CDR3 at positions that are known to be important for maintaining the binding site structure/contacts of the anti-CD22 antibody as determined by crystal structure and/or computer database analysis (*see* Chinese Pat. No. ZL200880024788.2, which is

incorporated herewith by reference). In some embodiments, no more than one to five conservative amino acid substitutions are made with the SM03 VL and/or VH CDR3 domains, or VL and/or VH CDR3 from irrelevant primate or human antibodies containing no more than one to five conservatively similar residues are used to replace the VL and/or VH CDR3 of SM03 or SM06. In some embodiments, no more than one to three conservative amino acid substitutions are made within the SM03 VL and/or VH CDR3 domains, or VL and/or VH CDR3 from irrelevant primate or human antibodies containing no more than one to three conservatively similar residues is used to replace the VL and/or VH CDR3 of SM03 or SM06.

[00107] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:7. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VH having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:8. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising: (a) a VL having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity sequence identity to a the amino acid sequence of SEQ ID NO:7; and (b) a VH having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity sequence identity to the amino acid sequence of SEQ ID NO:8.

[00108] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL, wherein the VL has at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO:7. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VL having at least 85% sequence identity to SEQ ID NO:7. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VL having at least 90% sequence identity to SEQ ID NO:7. In some embodiments, the anti-CD22 antibody or antigen-

binding fragment thereof has a VL having at least 95% sequence identity to SEQ ID NO:7. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VL having at least 98% sequence identity to SEQ ID NO:7. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL having the amino acid sequence of SEQ ID NO:7.

[00109] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VH, wherein the VH has at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO:8. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VH having at least 85% sequence identity to SEQ ID NO:8. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VH having at least 90% sequence identity to SEQ ID NO:8. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VH having at least 95% sequence identity to SEQ ID NO:8. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VH having at least 98% sequence identity to SEQ ID NO:8. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VH having the amino acid sequence of SEQ ID NO:8.

[00110] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:9. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VH having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:10. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising: (a) a VL having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO:9; and (b) a VH having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least

97%, at least 98%, at least 99%, or 100% sequence identity sequence identity to the amino acid sequence of SEQ ID NO:10.

[00111] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL, wherein the VL has at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO:9. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VL having at least 85% sequence identity to SEQ ID NO:9. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VL having at least 90% sequence identity to SEQ ID NO:9. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VL having at least 95% sequence identity to SEQ ID NO:9. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VL having at least 98% sequence identity to SEQ ID NO:9. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL having the amino acid sequence of SEQ ID NO:9.

[00112] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VH, wherein the VH has at least 80%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to SEQ ID NO:10. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VH having at least 85% sequence identity to SEQ ID NO:10. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VH having at least 90% sequence identity to SEQ ID NO:10. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VH having at least 95% sequence identity to SEQ ID NO:10. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof has a VH having at least 98% sequence identity to SEQ ID NO:10. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VH having the amino acid sequence of SEQ ID NO:10.

[00113] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL and a VH, wherein the VL and VH have the amino acid sequences of SEQ ID NOs:7 and 8, respectively. In some embodiments, the VL and VH have the amino acid sequences of SEQ ID NOs: 9 and 10, respectively.

[00114] The anti-CD22 antibodies or antigen-binding fragments thereof can comprise a combination of any VL disclosed herein and any VH disclosed herein. In some embodiments, the VL and VH are connected by a linker. The linker can be a flexible linker or a rigid linker. In some embodiments, the linker is a flexible linker. In some embodiments, the linker has the amino acid sequence of (GGGGS)_n, $n=3, 4$, or 5 (SEQ ID NO:19). In some embodiments, the linker has the amino acid sequence of GSAGSAAGSGEF (SEQ ID NO:38). In some embodiments, the linker has the amino acid sequence of KESGSVSSEQLAQFRSLD (SEQ ID NO:39). In some embodiments, the linker has the amino acid sequence of EGKSSSGSGSESKS (SEQ ID NO:40). In some embodiments, the linker has the amino acid sequence of SSGNSNANSRGPSFSSGLVPLSLRGSH (SEQ ID NO:41). In some embodiments, the linker has the amino acid sequence of GGGGGG (SEQ ID NO:42). In some embodiments, the linker is a rigid linker. In some embodiments, the linker has the amino acid sequence of (EAAAK)_n, $n=3, 4$, or 5 (SEQ ID NO:20). In some embodiments, the linker has the amino acid sequence of (PA)_nP, $n=1, 2, 3, 4$, or 5 (SEQ ID NO:21).

[00115] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising (a) a VL comprising VL CDRs 1, 2, and 3 from a VL having the amino acid sequence of SEQ ID NO:7 or 9; and/or (b) a VH comprising VH CDRs 1, 2, and 3 from a VH having the amino acid sequence of SEQ ID NO:8 or 10. In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising (a) a VL comprising VL CDRs 1, 2, and 3 from a VL having the amino acid sequence of SEQ ID NO:9; and/or (b) a VH comprising VH CDRs 1, 2, and 3 from a VH having the amino acid sequence of SEQ ID NO:10.

[00116] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL, wherein the VL comprises VL CDRs 1, 2, and 3 from a VL having the amino acid sequence of SEQ ID NO:7. In some embodiments, the VL comprises VL CDRs 1, 2, and 3 from a VL having the amino acid sequence of SEQ ID NO:9.

[00117] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VH, wherein the VH comprises VH CDRs 1, 2, and 3 from a VH having the amino acid sequence of SEQ ID NO:8. In some embodiments, the VH comprises VH CDRs 1, 2, and 3 from a VH having the amino acid sequence of SEQ ID NO:10.

[00118] In some embodiments, provided herein are antibodies or antigen-binding fragments thereof that specifically bind to CD22 comprising a VL and a VH, wherein the VL comprises VL CDR1, CDR2, and CDR3 from a VL having the amino acid sequence of SEQ ID NO:7, and the VH comprises VH CDR1, CDR2, and CDR3 from a VH having the amino acid sequence of SEQ ID

NO:8. In some embodiments, the VL comprises VL CDR1, CDR2, and CDR3 from a VL having the amino acid sequence of SEQ ID NO:9, and the VH comprises VH CDR1, CDR2, and CDR3 from a VH having the amino acid sequence of SEQ ID NO:10.

[00119] In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein is the antibody designated as SM03. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein has a VL from SM03. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein has a VH from SM03. The anti-CD22 antibody or antigen-binding fragment thereof provided herein can have both a VL and a VH from SM03. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein has a VL that comprises VL CDRs 1, 2, and 3 from the VL from SM03. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein has a VH that comprises VH CDRs 1, 2, and 3 from the VH from SM03. The anti-CD22 antibody or antigen-binding fragment thereof provided herein can have a VL comprising VL CDRs 1, 2, and 3 and a VH comprising VH CDRs 1, 2, and 3 from the VL and VH of SM03, respectively. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein is a variant of SM03. The SM03 variant can have a VL that is a variant of the VL of SM03 having up to about 5 amino acid substitutions, additions, and/or deletions in SEQ ID NO:7. The SM03 variant can have a VH that is a variant of the VH of SM03 having up to about 5 amino acid substitutions, additions, and/or deletions in SEQ ID NO:8. The amino acid substitutions, additions, and/or deletions can be in the VH CDRs or VL CDRs. In some embodiments, the amino acid substitutions, additions, and/or deletions are not in the CDRs. In some embodiments, the variant of SM03 has up to about 5 conservative amino acid substitutions. In some embodiments, the variant of SM03 has up to 3 conservative amino acid substitutions.

[00120] In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein is the antibody designated as SM06. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein has a VL from SM06. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein has a VH from SM06. The anti-CD22 antibody or antigen-binding fragment thereof provided herein can have both a VL and a VH from SM06. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein has a VL that comprises VL CDRs 1, 2, and 3 from the VL from SM06. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein has a VH that comprises VH CDRs 1, 2, and 3 from the VH from SM06. The anti-CD22 antibody or antigen-binding fragment thereof provided herein can have a VL comprising VL CDRs 1, 2, and 3 and a VH

comprising VH CDRs 1, 2, and 3 from the VL and VH of SM06, respectively. In some embodiments, the anti-CD22 antibody or antigen-binding fragment thereof provided herein is a variant of SM06. The SM06 variant can have a VL that is a variant of the VL of SM06 having up to about 5 amino acid substitutions, additions, and/or deletions in SEQ ID NO:9. The SM06 variant can have a VH that is a variant of the VH of SM06 having up to about 5 amino acid substitutions, additions, and/or deletions in SEQ ID NO:10. The amino acid substitutions, additions, and/or deletions can be in the VH CDRs or VL CDRs. In some embodiments, the amino acid substitutions, additions, and/or deletions are not in the CDRs. In some embodiments, the variant of SM06 has up to about 5 conservative amino acid substitutions. In some embodiments, the variant of SM06 has up to 3 conservative amino acid substitutions.

[00121] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments that can be used in methods disclosed herein comprise a VH or VL that has at least one framework (FR) region. The FR regions for VL can be from the V κ 10 murine germline family. In some embodiments, the FR1, FR2, FR3, and FR4 for VL can have the amino acid sequence of SEQ ID NOs:30, 31, 32, and 33, respectively (the SM03 VL framework sequences). In some embodiments, the FR region for VH can be from the VH5 murine germline family. In some embodiments, the FR1, FR2, FR3, and FR4 for VH can have the amino acid sequence of SEQ ID NOs:34, 35, 36, and 37, respectively (the SM03 VH framework sequences). In some embodiments, the FR1 region for VL can be from the V κ ID human germline family, the FR2 region for VL can be from the V κ 1 human germline family, the FR3 region for VL can be from the V κ 1 human germline family, and the FR4 region for VL can be from the V κ J1 human germline family. In some embodiments, the FR1, FR2, FR3, and FR4 for VL can have the amino acid sequences of SEQ ID NOs:22, 23, 24, and 25, respectively (the SM06 VL framework sequences). In some embodiments, the FR1 region for VH can be from the V μ 3 human germline family; the FR2 region regions for VH can be from the V μ 3 human germline family; the FR3 region for VH can be from the V μ 3 human germline family; and the FR4 region regions for VH can be from the V μ J5 human germline family. In some embodiments, the FR1, FR2, FR3, and FR4 for VH can have the amino acid sequences of SEQ ID NOs:26, 27, 28, and 29, respectively (the SM06 VH framework sequences).

[00122] The present disclosure further contemplates additional variants and equivalents that are substantially homologous to the recombinant, monoclonal, chimeric, humanized, and human antibodies, or antibody fragments thereof, described herein. In some embodiments, it is desirable to improve the binding affinity of the antibody. In some embodiments, it is desirable to modulate

biological properties of the antibody, including but not limited to, specificity, thermostability, expression level, effector function(s), glycosylation, immunogenicity, and/or solubility. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of an antibody, such as changing the number or position of glycosylation sites or altering membrane anchoring characteristics.

[00123] Variations can be a substitution, deletion, or insertion of one or more nucleotides encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the native antibody or polypeptide sequence. In some embodiments, amino acid substitutions are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *e.g.*, conservative amino acid replacements. Insertions or deletions can be in the range of about 1 to 5 amino acids. In some embodiments, the substitution, deletion, or insertion includes less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the parent molecule. In some embodiments, variations in the amino acid sequence that are biologically useful and/or relevant can be determined by systematically making insertions, deletions, or substitutions in the sequence and testing the resulting variant proteins for activity as compared to the parent protein.

[00124] In some embodiments, an anti-CD22 antibody or antigen-binding fragment thereof that can be used in methods provided herein is a chimeric antibody or antigen-binding fragment. In some embodiments, an anti-CD22 antibody or antigen-binding fragment thereof that can be used in methods provided herein is a humanized antibody or antigen-binding fragment. In some embodiments, an anti-CD22 antibody or antigen-binding fragment thereof comprises a VL CDR1, VL CDR2, VL CDR3, VH CDR1, VH CDR2, and/or VH CDR3 from an antibody or antigen-binding fragment described herein. In some embodiments, an anti-CD22 antibody or antigen-binding fragment thereof comprises a variant of an anti-CD22 antibody or antigen-binding fragment described herein. In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises one to 30 amino acid substitutions, additions, and/or deletions in the anti-CD22 antibody or antigen-binding fragment. In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises one to 25 amino acid substitutions, additions, and/or deletions in the anti-CD22 antibody or antigen-binding fragment. In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises one to 20 substitutions, additions, and/or deletions in the anti-CD22 antibody or antigen-binding fragment. In some embodiments, a variant of

an anti-CD22 antibody or antigen-binding fragment comprises one to 15 substitutions, additions, and/or deletions in the anti-CD22 antibody or antigen-binding fragment. In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises one to 10 substitutions, additions, and/or deletions in the anti-CD22 antibody or antigen-binding fragment. In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises one to five conservative amino acid substitutions, additions, and/or deletions in the anti-CD22 antibody or antigen-binding fragment. In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises one to three amino acid substitutions, additions, and/or deletions in the anti-CD22 antibody or antigen-binding fragment. In some embodiments, the amino acid substitutions, additions, and/or deletions are conservative amino acid substitutions. In some embodiments, the conservative amino acid substitution(s) is in a CDR of the antibody or antigen-binding fragment. In some embodiments, the conservative amino acid substitution(s) is not in a CDR of the antibody or antigen-binding fragment. In some embodiments, the conservative amino acid substitution(s) is in a framework region of the antibody or antigen-binding fragment.

[00125] It is known in the art that the constant region(s) of an antibody mediates several effector functions, and these effector functions can vary depending on the isotype of the antibody. For example, binding of the C1 component of complement to the Fc region of IgG or IgM antibodies (bound to antigen) activates the complement system. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can be involved in autoimmune hypersensitivity. In addition, the Fc region of an antibody can bind a cell expressing a Fc receptor (FcR). There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers many important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell cytotoxicity or ADCC), release of inflammatory mediators, placental transfer, and control of immunoglobulin production. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a human IgA antibody. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a human IgD antibody. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a human IgE antibody. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a

human IgG antibody. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a human IgM antibody. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a human IgG1 antibody. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a human IgG2 antibody. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a human IgG3 antibody. In some embodiments, anti-CD22 antibody or antigen-binding fragment described herein comprise at least one constant region of a human IgG4 antibody.

[00126] In some embodiments, at least one or more of the constant regions has been modified or deleted in the anti-CD22 antibody or antigen-binding fragment described herein. In some embodiments, the antibodies comprise modifications to one or more of the three heavy chain constant regions (CH1, CH2 or CH3) and/or to the light chain constant region (CL). In some embodiments, the heavy chain constant region of the modified antibodies comprises at least one human constant region. In some embodiments, the heavy chain constant region of the modified antibodies comprises more than one human constant region. In some embodiments, modifications to the constant region comprise additions, deletions, or substitutions of one or more amino acids in one or more regions. In some embodiments, one or more regions are partially or entirely deleted from the constant regions of the modified antibodies. In some embodiments, the entire CH2 domain has been removed from an antibody (Δ CH2 constructs). In some embodiments, a deleted constant region is replaced by a short amino acid spacer that provides some of the molecular flexibility typically imparted by the absent constant region. In some embodiments, a modified antibody comprises a CH3 domain directly fused to the hinge region of the antibody. In some embodiments, a modified antibody comprises a peptide spacer inserted between the hinge region and modified CH2 and/or CH3 domains.

[00127] In some embodiments, an anti-CD22 antibody or antigen-binding fragment comprises a Fc region. In some embodiments, the Fc region is fused via a hinge. The hinge can be an IgG1 hinge, an IgG2 hinge, or an IgG3 hinge. The amino acid sequences of the Fc region of human IgG1, IgG2, IgG3, and IgG4 are known to those of ordinary skill in the art. In some cases, Fc regions with amino acid variations have been identified in native antibodies. In some embodiments, the modified antibodies (*e.g.*, modified Fc region) provide for altered effector functions that, in turn, affect the biological profile of the antibody. For example, in some embodiments, the Fc regions of antibodies or antigen-binding fragments provided herein are modified to enhance their ability to cross the blood-brain barrier (BBB). In some embodiments, the deletion or inactivation (through point mutations or

other means) of a constant region reduces Fc receptor binding of the modified antibody as it circulates. In some embodiments, the constant region modifications reduce the immunogenicity of the antibody. In some embodiments, the constant region modifications increase the serum half-life of the antibody. In some embodiments, the constant region modifications reduce the serum half-life of the antibody. In some embodiments, the constant region modifications decrease or remove ADCC and/or complement dependent cytotoxicity (CDC) of the antibody. In some embodiments, specific amino acid substitutions in a human IgG1 Fc region with corresponding IgG2 or IgG4 residues reduce effector functions (*e.g.*, ADCC and CDC) in the modified antibody. In some embodiments, an antibody does not have one or more effector functions (*e.g.*, “effectorless” antibodies). In some embodiments, the antibody has no ADCC activity and/or no CDC activity. In some embodiments, the antibody does not bind an Fc receptor and/or complement factors. In some embodiments, the antibody has no effector function(s). In some embodiments, the constant region modifications increase or enhance ADCC and/or CDC of the antibody. In some embodiments, the constant region is modified to eliminate disulfide linkages or oligosaccharide moieties. In some embodiments, the constant region is modified to add/substitute one or more amino acids to provide one or more cytotoxin, oligosaccharide, or carbohydrate attachment sites. In some embodiments, an anti-CD22 antibody or antigen-binding fragment comprises a variant Fc region that is engineered with substitutions at specific amino acid positions as compared to a native Fc region. In some embodiments, an anti-CD22 antibody or antigen-binding fragment described herein comprises an IgG1 heavy chain constant region that comprises one or more amino acid substitutions selected from the group consisting of K214R, L234A, L235E, G237A, D356E, and L358M, per EU numbering. In some embodiments, the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, L234A, L235E, G237A, A330S, P331S, D356E, and L358M, per EU numbering. In some embodiments, the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, C226S, C229S, and P238S, per EU numbering. In some embodiments, the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of K214R, D356E, and L358M, per EU numbering. In some embodiments, the IgG1 heavy chain constant region comprises one or more amino acid substitutions selected from the group consisting of S131C, K133R, G137E, G138S, Q196K, I199T, N203D, K214R, C226S, C229S, and P238S, per EU numbering.

[00128] In some embodiments, variants can include addition of amino acid residues at the amino- and/or carboxyl-terminal end of the antibody or polypeptide. The length of additional amino acids residues can range from one residue to a hundred or more residues. In some embodiments, a variant comprises an N-terminal methionyl residue. In some embodiments, the variant comprises an additional polypeptide/protein (*e.g.*, Fc region) to create a fusion protein. In some embodiments, a variant is engineered to be detectable and may comprise a detectable label and/or protein (*e.g.*, a fluorescent tag or an enzyme).

[00129] Therapeutic antibodies can be re-engineered to facilitate transport across the BBB via various mechanism, including, for example, receptor mediated transcytosis, adsorptive transcytosis, carrier mediated transport, paracellular transport and diffusion. Among all, receptor mediated transcytosis (R.M.T.) has been widely explored to facilitate the entry of antibody. The use of R.M.T. includes transferrin receptor, insulin receptor, low density lipoprotein receptor (LDL receptor), CD98, TMEM50A, and other surface receptors on endothelial cells that can perform transcytosis upon binding. Antibody binding to the transferrin, insulin receptor, CD98 and TME50A can elicit transcytosis of the whole complex. In case of LDL receptor, binding of apolipoprotein can trigger the transcytosis process. Therefore, therapeutic antibody linked to an antibody fragment or apolipoprotein is proposed and validated to enhance BBB crossing in animal model.

[00130] As such, the anti-CD22 antibodies or antigen-binding fragments provided herein can be engineered to enhance its ability to cross BBB. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein are fused to a second antibody or antigen-binding fragment that binds to the transferrin, insulin receptor, CD98 or TME50A. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein can be fused to a second antibody or antigen-binding fragment that binds to transferrin. In some embodiments, for example, the anti-CD22 antibodies or antigen-binding fragment can be fused to a transferrin receptor binding Fab fragment. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein can be fused to a second antibody or antigen-binding fragment that binds to insulin receptor. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein can be fused to a second antibody or antigen-binding fragment that binds to CD98. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein can be fused to a second antibody or antigen-binding fragment that binds to TME50A. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein are fused to an apolipoprotein.

[00131] The variant antibodies or antigen-binding fragments described herein can be generated using methods known in the art, including but not limited to, site-directed mutagenesis, alanine scanning mutagenesis, and PCR mutagenesis.

[00132] In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment disclosed herein can retain the ability to bind to CD22 to a similar extent, the same extent, or to a higher extent, as the parent antibody or antigen-binding fragment. In some embodiments, the variant can be at least about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99% or more identical in amino acid sequence to the parent antibody or antigen-binding fragment. In certain embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises the amino acid sequence of the parent anti-CD22 antibody or antigen-binding fragment with one or more conservative amino acid substitution. Conservative amino acid substitutions are known in the art and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties.

[00133] In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises the amino acid sequence of the parent antibody or antigen-binding fragment with one or more non-conservative amino acid substitutions. In some embodiments, a variant of an anti-CD22 antibody or antigen-binding fragment comprises the amino acid sequence of the parent binding antibody or antigen-binding fragment with one or more non-conservative amino acid substitution, wherein the one or more non-conservative amino acid substitutions do not interfere with or inhibit one or more biological activities of the variant (*e.g.*, CD22 binding). In certain embodiments, the one or more conservative amino acid substitutions and/or the one or more non-conservative amino acid substitutions can enhance a biological activity of the variant, such that the biological activity of the functional variant is increased as compared to the parent binding moiety.

[00134] In some embodiments, anti-CD22 antibodies or antigen-binding fragments described herein are chemically modified naturally or by intervention. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments have been chemically modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, and/or linkage to a cellular ligand or other protein. Any of numerous chemical modifications can be carried out by known techniques. The anti-CD22 antibodies or antigen-binding fragments can comprise one or more analogs of an amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art.

[00135] The anti-CD22 antibodies or antigen-binding fragments of the present disclosure can be analyzed for their physical, chemical and/or biological properties by various methods known in the art. In some embodiments, an anti-CD22 antibody is tested for its ability to bind to CD22 (*e.g.*, human CD22). Binding assays include, but are not limited to, surface plasmon resonance (*e.g.*, Biacore), ELISA, and FACS. In some embodiments, the dissociation constant of the binding agent (*e.g.*, an antibody) for CD22 is the dissociation constant determined by surface plasmon resonance (*e.g.*, Biacore). In addition, antibodies can be evaluated for solubility, stability, thermostability, viscosity, expression levels, expression quality, and/or purification efficiency.

[00136] Both SM03 and SM06 bind human CD22 with a dissociation constant (K_D) of about 1.2 nM. In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein binds to CD22 (*e.g.*, human CD22) with a dissociation constant (K_D) of about 100 nM or less, about 40 nM or less, about 20 nM or less, about 10 nM or less, about 1 nM or less, about 0.1 nM or less, 50 pM or less, 10 pM or less, or 1 pM or less. In some embodiments, the K_D is about 20 nM or less. In some embodiments, the K_D is about 10 nM or less. In some embodiments, the K_D is about 5 nM or less. In some embodiments, the K_D is about 2 nM or less. In some embodiments, the K_D is about 1.5 nM or less. In some embodiments, the K_D is about 1 nM or less. In some embodiments, the K_D is about 0.5 nM or less. In some embodiments, the K_D is about 0.1 nM or less. In some embodiments, the K_D is about 50 pM or less. In some embodiments, the K_D is about 10 pM or less.

[00137] In some embodiments, an anti-CD22 antibody or antigen-binding fragment binds to CD22 (*e.g.*, human CD22) with a K_D within the range of 0.1-1 nM, 0.5-5 nM, 1-10 nM, 1-5 nM, 5-50 nM, 10-100 nM, or 50-500 nM. In some embodiments, the K_D is within the range of 0.1-1 nM. In some embodiments, the K_D is within the range of 0.5-5 nM. In some embodiments, the K_D is within the range of 1-10 nM. In some embodiments, the K_D is within the range of 1-5 nM. In some embodiments, the K_D is within the range of 5-50 nM. In some embodiments, the K_D is within the range of 10-100 nM. In some embodiments, the K_D is within the range of 50-500 nM.

[00138] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein binds to CD22 (*e.g.*, human CD22) with an association constant (K_A) of about $0.8 \times 10^9 \text{ M}^{-1}$. In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein binds to CD22 (*e.g.*, human CD22) with a K_A of about $1 \times 10^6 \text{ M}^{-1}$ or more, about $1 \times 10^7 \text{ M}^{-1}$ or more, about $1 \times 10^8 \text{ M}^{-1}$ or more, about $5 \times 10^8 \text{ M}^{-1}$ or more, about $8 \times 10^8 \text{ M}^{-1}$ or more, about $1 \times 10^9 \text{ M}^{-1}$ or more, about $5 \times 10^9 \text{ M}^{-1}$ or more, about $1 \times 10^{10} \text{ M}^{-1}$ or more, about $5 \times 10^{10} \text{ M}^{-1}$ or more, about $1 \times 10^{11} \text{ M}^{-1}$ or more, about $5 \times 10^{11} \text{ M}^{-1}$ or more, or about $1 \times 10^{12} \text{ M}^{-1}$ or more. In

some embodiments, the K_A is about $1 \times 10^7 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $5 \times 10^7 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $1 \times 10^8 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $5 \times 10^8 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $8 \times 10^8 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $1 \times 10^9 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $5 \times 10^9 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $1 \times 10^{10} \text{ M}^{-1}$ or more.

[00139] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein binds to CD22 (*e.g.*, human CD22) with a K_A within the range of about 1×10^6 - $1 \times 10^7 \text{ M}^{-1}$, 5×10^6 - $5 \times 10^7 \text{ M}^{-1}$, 1×10^7 - $1 \times 10^8 \text{ M}^{-1}$, 5×10^7 - $5 \times 10^8 \text{ M}^{-1}$, 1×10^8 - $5 \times 10^8 \text{ M}^{-1}$, 1×10^8 - $1 \times 10^9 \text{ M}^{-1}$, 5×10^8 - $1 \times 10^9 \text{ M}^{-1}$, 5×10^8 - $5 \times 10^9 \text{ M}^{-1}$, 1×10^9 - $1 \times 10^{10} \text{ M}^{-1}$, 5×10^9 - $5 \times 10^{10} \text{ M}^{-1}$, 1×10^{10} - $1 \times 10^{11} \text{ M}^{-1}$, 5×10^{10} - $5 \times 10^{11} \text{ M}^{-1}$, 1×10^{11} - $1 \times 10^{12} \text{ M}^{-1}$, or 5×10^{11} - $5 \times 10^{12} \text{ M}^{-1}$. In some embodiments, the K_A is within the range of about 1×10^6 - $1 \times 10^7 \text{ M}^{-1}$. In some embodiments, the K_A is within the range of about 1×10^7 - $1 \times 10^8 \text{ M}^{-1}$. In some embodiments, the K_A is within the range of about 1×10^8 - $1 \times 10^9 \text{ M}^{-1}$. In some embodiments, the K_A is within the range of about 5×10^8 - $1 \times 10^9 \text{ M}^{-1}$. In some embodiments, the K_A is within the range of about 5×10^8 - $5 \times 10^9 \text{ M}^{-1}$. In some embodiments, the K_A is within the range of about 1×10^9 - $1 \times 10^{10} \text{ M}^{-1}$.

[00140] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein dissociates from human CD22 with a k_d of 0.0685 RU s^{-1} or less, or 0.0137 RU s^{-1} or less, as determined by SPR (*e.g.*, BIAcore). In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein dissociates from human CD22 with a k_d of about 0.5 RU s^{-1} or less, about 0.2 RU s^{-1} or less, about 0.1 RU s^{-1} or less, about 0.08 RU s^{-1} or less, about 0.06 RU s^{-1} or less, about 0.05 RU s^{-1} or less, about 0.04 RU s^{-1} or less, about 0.03 RU s^{-1} or less, about 0.02 RU s^{-1} or less, about 0.01 RU s^{-1} or less, about 0.008 RU s^{-1} or less, about 0.005 RU s^{-1} or less, or about 0.001 RU s^{-1} or less. In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein dissociates from human CD22 with a k_d of about 0.5 RU s^{-1} or less. In some embodiments, the k_d is about 0.2 RU s^{-1} or less. In some embodiments, the k_d is about 0.1 RU s^{-1} or less. In some embodiments, the k_d is about 0.08 RU s^{-1} or less. In some embodiments, the k_d is about 0.06 RU s^{-1} or less. In some embodiments, the k_d is about 0.05 RU s^{-1} or less. In some embodiments, the k_d is about 0.02 RU s^{-1} or less. In some embodiments, the k_d is about 0.01 RU s^{-1} or less. In some embodiments, the k_d is about 0.005 RU s^{-1} or less.

[00141] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein dissociates from human CD22 with a k_d with the range of 0.001 - 0.5 RU s^{-1} , 0.001 - 0.1 RU s^{-1} , 0.001 - 0.05 RU s^{-1} , 0.005 - 0.5 RU s^{-1} , 0.005 - 0.1 RU s^{-1} , 0.01 - 0.5 RU s^{-1} , 0.01 - 0.1 RU s^{-1} , or 0.01 - 0.05 RU s^{-1} . In some embodiments, the k_d is within the range of 0.005 - 0.05 RU s^{-1} .

In some embodiments, the k_d is within the range of 0.01-0.1 RU s^{-1} . In some embodiments, the k_d is within the range of 0.005-0.1 RU s^{-1} . In some embodiments, the k_d is within the range of 0.01-0.5 RU s^{-1} . In some embodiments, the k_d is within the range of 0.01-0.1 RU s^{-1} .

[00142] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein associates with human CD22 with a k_a of $1.13 \times 10^7 \text{ RU S}^{-1}$ or higher, as determined by SPR (*e.g.*, BIAcore). In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein associates with human CD22 with a k_a of about $1.0 \times 10^5 \text{ RU S}^{-1}$ or higher, about $5.0 \times 10^5 \text{ RU S}^{-1}$ or higher, about $1.0 \times 10^6 \text{ RU S}^{-1}$ or higher, about $5.0 \times 10^6 \text{ RU S}^{-1}$ or higher, about $1.0 \times 10^7 \text{ RU S}^{-1}$ or higher, about $2.0 \times 10^7 \text{ RU S}^{-1}$ or higher, about $3 \times 10^7 \text{ RU S}^{-1}$ or higher, about $4.0 \times 10^7 \text{ RU S}^{-1}$ or higher, about $5.0 \times 10^7 \text{ RU S}^{-1}$ or higher, about $1.0 \times 10^8 \text{ RU S}^{-1}$ or higher, about $5.0 \times 10^8 \text{ RU S}^{-1}$ or higher, or about $1.0 \times 10^9 \text{ RU S}^{-1}$ or higher. In some embodiments, the k_a is about $1.0 \times 10^6 \text{ RU S}^{-1}$ or higher. In some embodiments, the k_a is about $5.0 \times 10^6 \text{ RU S}^{-1}$ or higher. In some embodiments, the k_a is about $1.0 \times 10^7 \text{ RU S}^{-1}$ or higher. In some embodiments, the k_a is about $2.0 \times 10^7 \text{ RU S}^{-1}$ or higher. In some embodiments, the k_a is about $5.0 \times 10^7 \text{ RU S}^{-1}$ or higher. In some embodiments, the k_a is about $1.0 \times 10^8 \text{ RU S}^{-1}$ or higher. In some embodiments, the k_a is about $1.0 \times 10^9 \text{ RU S}^{-1}$ or higher.

[00143] In some embodiments, the k_a is within the range of $1.0 \times 10^5 - 1.0 \times 10^6 \text{ RU S}^{-1}$, $1.0 \times 10^6 - 1.0 \times 10^7 \text{ RU S}^{-1}$, $5.0 \times 10^6 - 5.0 \times 10^7 \text{ RU S}^{-1}$, $1.0 \times 10^7 - 5.0 \times 10^7 \text{ RU S}^{-1}$, $1.0 \times 10^7 - 1.0 \times 10^8 \text{ RU S}^{-1}$, or $1.0 \times 10^8 - 1.0 \times 10^9 \text{ RU S}^{-1}$. In some embodiments, the k_a is within the range of $1.0 \times 10^6 - 1.0 \times 10^7 \text{ RU S}^{-1}$. In some embodiments, the k_a is within the range of $5.0 \times 10^6 - 5.0 \times 10^7 \text{ RU S}^{-1}$. In some embodiments, the k_a is within the range of $1.0 \times 10^7 - 5.0 \times 10^7 \text{ RU S}^{-1}$. In some embodiments, the k_a is within the range of $1.0 \times 10^7 - 1.0 \times 10^8 \text{ RU S}^{-1}$. In some embodiments, the k_a is within the range of $1.0 \times 10^8 - 1.0 \times 10^9 \text{ RU S}^{-1}$.

[00144] Epitope mapping is a method of identifying the binding site, region, or epitope on a target protein where an antibody binds. A variety of methods are known in the art for mapping epitopes on target proteins. These methods include mutagenesis, including but not limited to, shotgun mutagenesis, site-directed mutagenesis, and alanine scanning; domain or fragment scanning; peptide scanning (*e.g.*, Pepscan technology); display methods (*e.g.*, phage display, microbial display, and ribosome/mRNA display); methods involving proteolysis and mass spectroscopy; and structural determination (*e.g.*, X-ray crystallography and NMR). In some embodiments, anti-CD22 antibodies or antigen-binding fragments described herein are characterized by assays including, but not limited to, N-terminal sequencing, amino acid analysis, HPLC, mass spectrometry, ion exchange chromatography, and papain digestion.

[00145] As described in Leung *et al.* (2015) *Mabs* 7(1):66-76; Zhao *et al.* (2014) *PLOS ONE* 9(5): e96697; US Pat. No. US 9371396B2; and Chinese Pat. No. ZL201210286457.4; which are incorporated herein in their entirety by reference, SM03 and SM06 bind to the domain 2 of human CD22 with high affinity, specifically at the discontinuous conformational epitope encompassing the sequence $_{161}\text{CLLNFSYGYPIQ}_{173}$ (SEQ ID NO:17) and $_{198}\text{VFTRSELKFSPQWSHHGKIVTC}_{219}$ (SEQ ID NO:18) with affinity (K_A) in the range of $0.82 \times 10^9 \text{ M}^{-1}$. Binding of this conformational epitope can induce the rapid internalization of CD22 (*e.g.*, 50% of the surface CD22 can be internalized within 10 minutes), promote the *cis-trans* conversion of the 2,6-sialic acid binding of CD22, and restores immunotolerance. Also, both SM03 and SM06 can bind anti-idiotypic antibody against the antigen binding site (ABS) of SM03 and SM06 (*e.g.*, LRID) with an EC_{50} in the range of $79.9 \pm 27.6 \text{ ng/ml}$.

[00146] Accordingly, the anti-CD22 antibodies or antigen-binding fragments that can be used in the methods disclosed herein can bind an epitope that does not sterically interfere CD22's interaction with $\text{A}\beta$. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments bind a specific conformational epitope that does not sterically interfere with CD22's interaction with $\text{A}\beta$. In some embodiments, the epitope comprises $_{161}\text{CLLNFSYGYPIQ}_{173}$ (SEQ ID NO:17). In some embodiments, the epitope comprises $_{198}\text{VFTRSELKFSPQWSHHGKIVTC}_{219}$ (SEQ ID NO:18). In some embodiments, the epitope is conformational and comprises both $_{161}\text{CLLNFSYGYPIQ}_{173}$ (SEQ ID NO:17) and $_{198}\text{VFTRSELKFSPQWSHHGKIVTC}_{219}$ (SEQ ID NO:18).

[00147] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments that can be used in the methods disclosed herein can bind this conformational epitope with a K_A of about $0.8 \times 10^9 \text{ M}^{-1}$. In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein binds this conformational epitope with a K_A of about $1 \times 10^7 \text{ M}^{-1}$ or more, about $1 \times 10^8 \text{ M}^{-1}$ or more, about $5 \times 10^8 \text{ M}^{-1}$ or more, about $1 \times 10^9 \text{ M}^{-1}$ or more, about $5 \times 10^9 \text{ M}^{-1}$ or more, about $1 \times 10^{10} \text{ M}^{-1}$ or more, about $5 \times 10^{10} \text{ M}^{-1}$ or more, about $1 \times 10^{11} \text{ M}^{-1}$ or more, about $5 \times 10^{11} \text{ M}^{-1}$ or more, or about $1 \times 10^{12} \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $1 \times 10^7 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $5 \times 10^7 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $1 \times 10^8 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $5 \times 10^8 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $8 \times 10^8 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $1 \times 10^9 \text{ M}^{-1}$ or more. In some embodiments, the K_A is about $5 \times 10^9 \text{ M}^{-1}$ or more.

[00148] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein binds this conformational epitope with a K_A within the range of about

1×10^6 - 1×10^7 M^{-1} , 5×10^6 - 5×10^7 M^{-1} , 1×10^7 - 1×10^8 M^{-1} , 5×10^7 - 5×10^8 M^{-1} , 1×10^8 - 5×10^8 M^{-1} , 1×10^8 - 1×10^9 M^{-1} , 5×10^8 - 1×10^9 M^{-1} , 5×10^8 - 5×10^9 M^{-1} , 1×10^9 - 1×10^{10} M^{-1} , 5×10^9 - 5×10^{10} M^{-1} , 1×10^{10} - 1×10^{11} M^{-1} , 5×10^{10} - 5×10^{11} M^{-1} , 1×10^{11} - 1×10^{12} M^{-1} , or 5×10^{11} - 5×10^{12} M^{-1} . In some embodiments, the K_A is within the range of about 1×10^6 - 1×10^7 M^{-1} . In some embodiments, the K_A is within the range of about 1×10^6 - 1×10^7 M^{-1} . In some embodiments, the K_A is within the range of about 1×10^7 - 1×10^8 M^{-1} . In some embodiments, the K_A is within the range of about 1×10^8 - 1×10^9 M^{-1} . In some embodiments, the K_A is within the range of about 5×10^8 - 1×10^9 M^{-1} . In some embodiments, the K_A is within the range of about 5×10^8 - 5×10^9 M^{-1} . In some embodiments, the K_A is within the range of about 1×10^9 - 1×10^{10} M^{-1} .

[00149] In some embodiments, an anti-CD22 antibody or antigen-binding fragment used in the methods disclosed herein binds the anti-idiotypic antibody with high affinity (described in, *e.g.*, Leung 2015 *supra*; Zhao 2014 *supra*). The anti-idiotypic antibody has a VL and VH, each having the amino acid sequences of SEQ ID NOs:13 and 14, respectively (“LRID”). In some embodiments, anti-CD22 antibodies or antigen-binding fragments used in the methods disclosed herein bind LRID with EC_{50} of about 0.40 $\mu g/ml$ or less. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments can bind LRID with an EC_{50} of about 0.08 $\mu g/ml$. In some embodiments, the EC_{50} is about 5.0 $\mu g/ml$, about 2.0 $\mu g/ml$, about 1.0 $\mu g/ml$, about 0.8 $\mu g/ml$, about 0.5 $\mu g/ml$, about 0.2 $\mu g/ml$, about 0.1 $\mu g/ml$, about 0.08 $\mu g/ml$, about 0.05 $\mu g/ml$, about 0.02 $\mu g/ml$, about 0.01 $\mu g/ml$, about 0.008 $\mu g/ml$, about 0.005 $\mu g/ml$, or about 0.001 $\mu g/ml$. In some embodiments, the EC_{50} is about 2.0 $\mu g/ml$. In some embodiments, the EC_{50} is about 1.0 $\mu g/ml$. In some embodiments, the EC_{50} is about 0.8 $\mu g/ml$. In some embodiments, the EC_{50} is about 0.5 $\mu g/ml$. In some embodiments, the EC_{50} is about 0.2 $\mu g/ml$. In some embodiments, the EC_{50} is about 0.1 $\mu g/ml$. In some embodiments, the EC_{50} is about 0.08 $\mu g/ml$. In some embodiments, the EC_{50} is about 0.05 $\mu g/ml$. In some embodiments, the EC_{50} is about 0.01 $\mu g/ml$.

[00150] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods disclosed herein bind LRID with an EC_{50} with the range of 0.01-5 $\mu g/ml$, 0.01-1 $\mu g/ml$, 0.01-0.5 $\mu g/ml$, 0.05-5 $\mu g/ml$, 0.05-1 $\mu g/ml$, 0.05-0.5 $\mu g/ml$, 0.1-5 $\mu g/ml$, or 0.1-1 $\mu g/ml$. In some embodiments, the EC_{50} is with the range of 0.01-1 $\mu g/ml$. In some embodiments, the EC_{50} is with the range of 0.01-0.5 $\mu g/ml$. In some embodiments, the EC_{50} is with the range of 0.05-1 $\mu g/ml$. In some embodiments, the EC_{50} is with the range of 0.05-0.5 $\mu g/ml$.

[00151] In some embodiments, provided herein are also antibodies or antigen-binding fragments that compete with the antibody or antigen-binding fragment provided above for binding to CD22 (*e.g.*, human CD22). Antibodies that “compete with another antibody for binding to a target” refer to

antibodies that inhibit (partially or completely) the binding of the other antibody to the target. Whether two antibodies compete with each other for binding to a target, *i.e.*, whether and to what extent one antibody inhibits the binding of the other antibody to a target, can be determined using known competition experiments, *e.g.*, BIACORE® surface plasmon resonance (SPR) analysis. In some embodiments, an anti-CD22 antibody or antigen-binding fragment competes with, and inhibits binding of another antibody or antigen-binding fragment to CD22 (*e.g.*, human CD22) by at least 50%, 60%, 70%, 80%, 90% or 100%. Competition assays can be conducted as described, for example, in Ed Harlow and David Lane, Cold Spring Harb Protoc; 2006; doi: 10.1016/pdb.prot4277 or in Chapter 11 of “Using Antibodies” by Ed Harlow and David Lane, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA 1999.

[00152] In some embodiments, provided herein are antibodies or antigen-binding fragments that compete with SM03 for binding to human CD22. In some embodiments, provided herein are antibodies or antigen-binding fragments that compete with SM06 for binding to human CD22. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods disclosed herein compete with radiolabeled I¹²⁵-SM03 binding to native CD22 on Ramos cell, a human Burkitt's lymphoma cell line, with an EC₅₀ of about 5.01 µg/ml or less, or about 1.02 µg/ml or less. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods disclosed herein compete with radiolabeled I¹²⁵-SM03 binding to native CD22 on Ramos cell, a human Burkitt's lymphoma cell line, with an EC₅₀ of about 5.01 µg/ml or less, or about 1.02 µg/ml. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods disclosed herein compete with radiolabeled I¹²⁵-SM03 binding to native CD22 on Ramos cell with an EC₅₀ of about 0.1 µg/ml or less, about 0.2 µg/ml or less, about 0.5 µg/ml or less, about 0.8 µg/ml or less, about 1 µg/ml or less, about 2 µg/ml or less, about 5.0 µg/ml or less, about 8 µg/ml or less, about 10 µg/ml or less, or about 50 µg/ml or less. In some embodiments, the EC₅₀ is about 0.1 µg/ml or less. In some embodiments, the EC₅₀ is about 0.5 µg/ml or less. In some embodiments, the EC₅₀ is about 1 µg/ml or less. In some embodiments, the EC₅₀ is about 2 µg/ml or less. In some embodiments, the EC₅₀ is about 5 µg/ml or less. In some embodiments, the EC₅₀ is about 10 µg/ml or less. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods disclosed herein compete with radiolabeled I¹²⁵-SM03 binding to native CD22 on Ramos cell with an EC₅₀ within the range of about 0.1-50 µg/ml, about 0.1-10 µg/ml, about 0.1-5 µg/ml, about 0.5-10 µg/ml, about 0.5-5 µg/ml, about 1-10 µg/ml or about 1-5 µg/ml. In some embodiments, the EC₅₀ is within the range of about 0.1-50 µg/ml. In some embodiments, the EC₅₀ is within the

range of about 0.1-10 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 0.1-5 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 0.5-10 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 0.5-5 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 1-10 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 1-5 $\mu\text{g/ml}$.

[00153] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods disclosed herein compete with radiolabeled I^{125} -SM03 binding to native CD22 on a microglia cell (*e.g.*, HMC-3 cell) with an EC_{50} of about 0.1 $\mu\text{g/ml}$ or less, about 0.2 $\mu\text{g/ml}$ or less, about 0.5 $\mu\text{g/ml}$ or less, about 0.8 $\mu\text{g/ml}$ or less, about 1 $\mu\text{g/ml}$ or less, about 2 $\mu\text{g/ml}$ or less, about 5.0 $\mu\text{g/ml}$ or less, about 8 $\mu\text{g/ml}$ or less, about 10 $\mu\text{g/ml}$ or less, or about 50 $\mu\text{g/ml}$ or less. In some embodiments, the EC_{50} is about 0.1 $\mu\text{g/ml}$ or less. In some embodiments, the EC_{50} is about 0.2 $\mu\text{g/ml}$ or less. In some embodiments, the EC_{50} is about 0.5 $\mu\text{g/ml}$ or less. In some embodiments, the EC_{50} is about 1 $\mu\text{g/ml}$ or less. In some embodiments, the EC_{50} is about 2 $\mu\text{g/ml}$ or less. In some embodiments, the EC_{50} is about 5 $\mu\text{g/ml}$ or less. In some embodiments, the EC_{50} is about 10 $\mu\text{g/ml}$ or less. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments used in the methods disclosed herein compete with radiolabeled I^{125} -SM03 binding to native CD22 on a microglia cell (*e.g.*, HMC-3 cell) with an EC_{50} within the range of about 0.1-50 $\mu\text{g/ml}$, about 0.1-10 $\mu\text{g/ml}$, about 0.1-5 $\mu\text{g/ml}$, about 0.5-10 $\mu\text{g/ml}$, about 0.5-5 $\mu\text{g/ml}$, about 1-10 $\mu\text{g/ml}$ or about 1-5 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 0.1-50 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 0.1-10 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 0.1-5 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 0.5-10 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 0.5-5 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 1-10 $\mu\text{g/ml}$. In some embodiments, the EC_{50} is within the range of about 1-5 $\mu\text{g/ml}$.

[00154] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein can induce the internalization of about 20%, about 30%, about 40%, about 50%, about 60%, about 70% of the surface CD22 within 10 minutes (min) upon contact with the cell (*e.g.*, B cell, or microglia cell). In some embodiments, the internalization rate is about 30% of the surface CD22 within 10 min. In some embodiments, the internalization rate is about 40% of the surface CD22 within 10 min. In some embodiments, the internalization rate is about 50% of the surface CD22 within 10 min. In some embodiments, the internalization rate is about 60% of the surface CD22 within 10 min. In some embodiments, the internalization rate is about 70% of the surface CD22 within 10 min. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments

provided herein can induce the internalization of about 20% to 70%, about 30% to 70%, about 40% to 70%, about 30% to 60%, or about 40% to 60% of the surface CD22 within 10 min upon contact. In some embodiments, the internalization rate is about 20% to 70% of the surface CD22 within 10 min. In some embodiments, the internalization rate is about 40% to 70% of the surface CD22 within 10 min. In some embodiments, the internalization rate is about 30% to 60% of the surface CD22 within 10 min. In some embodiments, the internalization rate is about 40% to 60% of the surface CD22 within 10 min.

[00155] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein can induce the internalization of about 50% of the surface CD22 within about 2 min, about 5 min, about 10 min, about 15 min, about 20 min, about 30 min, or about 1 hour upon contact with the cell (*e.g.*, B cell, or microglia cell). In some embodiments, 50% of the surface CD22 is internalized within 2 min. In some embodiments, 50% of the surface CD22 is internalized within 5 min. In some embodiments, 50% of the surface CD22 is internalized within 10 min. In some embodiments, 50% of the surface CD22 is internalized within 15 min. In some embodiments, 50% of the surface CD22 is internalized within 20 min. In some embodiments, 50% of the surface CD22 is internalized within 30 min.

[00156] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein can induce the internalization of A β at a rate of about 0.1×10^{-6} pg/s/cell, about 0.2×10^{-6} pg/s/cell, about 0.5×10^{-6} pg/s/cell, about 0.8×10^{-6} pg/s/cell, about 1.0×10^{-6} pg/s/cell, about 2.0×10^{-6} pg/s/cell, about 4.0×10^{-6} , about 5.0×10^{-6} pg/s/cell, about 6.0×10^{-6} pg/s/cell, about 7.0×10^{-6} pg/s/cell, about 8.0×10^{-6} pg/s/cell, about 1.0×10^{-5} pg/s/cell, about 2.0×10^{-5} pg/s/cell, about 5.0×10^{-5} pg/s/cell, or about 8.0×10^{-5} pg/s/cell. In some embodiments, the rate is about 0.2×10^{-6} pg/s/cell. In some embodiments, the rate is about 0.5×10^{-6} pg/s/cell. In some embodiments, the rate is about 0.8×10^{-6} pg/s/cell. In some embodiments, the rate is about 1.0×10^{-6} pg/s/cell. In some embodiments, the rate is about 2.0×10^{-6} pg/s/cell. In some embodiments, the rate is about 4.0×10^{-6} pg/s/cell. In some embodiments, the rate is about 6.0×10^{-6} pg/s/cell. In some embodiments, the rate is about 1.0×10^{-5} pg/s/cell. In some embodiments, the rate is about 5.0×10^{-5} pg/s/cell. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments provided herein can induce the internalization of A β at a rate of about $0.1-50 \times 10^{-6}$ pg/s/cell, about $0.1-20 \times 10^{-6}$ pg/s/cell, about $0.1-10 \times 10^{-6}$ pg/s/cell, about $0.5-50 \times 10^{-6}$ pg/s/cell, about $0.5-20 \times 10^{-6}$ pg/s/cell, about $0.5-10 \times 10^{-6}$ pg/s/cell, about $1-50 \times 10^{-6}$ pg/s/cell, about $1-20 \times 10^{-6}$ pg/s/cell, about $1-10 \times 10^{-6}$ pg/s/cell, or about $1-6 \times 10^{-6}$ pg/s/cell. In some embodiments, the rate is about $0.1-50 \times 10^{-6}$ pg/s/cell. In some embodiments, the rate is about $0.5-50 \times 10^{-6}$ pg/s/cell. In some embodiments, the rate is about $0.5-10 \times 10^{-6}$ pg/s/cell. In some

embodiments, the rate is about $1-50 \times 10^{-6}$ pg/s/cell. In some embodiments, the rate is about. In some embodiments, the rate is about $1-10 \times 10^{-6}$ pg/s/cell. In some embodiments, the rate is about. In some embodiments, the rate is about $1-6 \times 10^{-6}$ pg/s/cell.

[00157] In some embodiments, anti-CD22 antibodies or antigen-binding fragments provided herein can be derivatized or linked to another functional molecule (*e.g.*, another peptide or protein) and used in methods disclosed herein. Accordingly, in some embodiments, the antibodies and antigen-binding fragments used in methods disclosed herein include derivatized and otherwise modified forms of the human anti-CD22 antibodies described herein, including immunoadhesion molecules. For example, the antibodies and antigen-binding fragments can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or introduction of an artificial amino acid/functional group suitable for site-specific conjugation) to one or more other molecular entities, such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antigen-binding fragment with another molecule (such as a streptavidin core region or a polyhistidine tag).

[00158] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, *e.g.*, to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (*e.g.*, m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (*e.g.*, disuccinimidyl suberate). Such linkers are available from Thermo Scientific, Waltham, MA.

[00159] In some embodiments, an anti-CD22 antibody or antigen-binding fragment is conjugated to a cytotoxic agent or moiety. In some embodiments, an anti-CD22 antibody or antigen-binding fragment is conjugated to a cytotoxic agent to form an ADC (antibody-drug conjugate). In some embodiments, the cytotoxic moiety is a chemotherapeutic agent including, but not limited to, methotrexate, adriamycin/doxorubicin, melphalan, mitomycin C, chlorambucil, duocarmycin, daunorubicin, pyrrolbenzodiazepines (PBDs), or other intercalating agents. In some embodiments, the cytotoxic moiety is a microtubule inhibitor including, but not limited to, auristatins, maytansinoids (*e.g.*, DM1 and DM4), and tubulysins. In some embodiments, the cytotoxic moiety is an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof, including, but not limited to, diphtheria A chain, non-binding active fragments of diphtheria toxin, exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcumin, croton, Saponaria officinalis inhibitor, gelonin, mitogellin, restrictocin,

phenomycin, enomycin, and the tricothecenes. In some embodiments, an antibody is conjugated to one or more small molecule toxins, such as calicheamicins, maytansinoids, tricothecenes, and CC1065.

[00160] In some embodiments, an anti-CD22 antibody or antigen-binding fragment described herein is conjugated to a detectable substance or molecule that allows the agent to be used for diagnosis and/or detection. A detectable substance can also include, but is not limited to, enzymes, such as horseradish peroxidase, alkaline phosphatase, glucose oxidase, beta-galactosidase, and acetylcholinesterase; prosthetic groups, such as biotin and flavine(s); fluorescent materials, such as, umbelliferone, fluorescein, fluorescein isothiocyanate (FITC), rhodamine, tetramethylrhodamine isothiocyanate (TRITC), dichlorotriazinylamine fluorescein, dansyl chloride, cyanine (Cy3), 5-dimethylamine-1-naphthalenesulfonyl chloride, and phycoerythrin; bioluminescent materials, such as luciferase; radioactive materials, such as ^{212}Bi , ^{14}C , ^{57}Co , ^{51}Cr , ^{67}Cu , ^{18}F , ^{68}Ga , ^{67}Ga , ^{153}Gd , ^{159}Gd , ^{68}Ge , ^3H , ^{166}Ho , ^{131}I , ^{125}I , ^{123}I , ^{121}I , ^{115}In , ^{113}In , ^{112}In , ^{111}In , ^{140}La , ^{177}Lu , ^{54}Mn , ^{99}Mo , ^{32}P , ^{103}Pd , ^{149}Pm , ^{142}Pr , ^{186}Re , ^{188}Re , ^{105}Rh , ^{97}Ru , ^{35}S , ^{47}Sc , ^{75}Se , ^{153}Sm , ^{113}Sn , ^{117}Sn , ^{85}Sr , $^{99\text{m}}\text{Tc}$, ^{201}Ti , ^{133}Xe , ^{90}Y , ^{69}Yb , ^{175}Yb , ^{65}Zn ; positron emitting metals; and magnetic metal ions positron emitting metals; and magnetic metal ions.

[00161] An anti-CD22 antibody or antigen-binding fragment described herein can be attached to a solid support. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride, or polypropylene. In some embodiments, an immobilized anti-CD22 antibody or antigen-binding fragment is used in an immunoassay. In some embodiments, an immobilized anti-CD22 antibody or antigen-binding fragment is used in purification of the target antigen (*e.g.*, human CD22).

5.2.2 Additional CD22 antibodies

[00162] Disclosed herein are methods of promoting removal of A β plaque, methods of reducing neuroinflammation, methods of preventing synaptic phagocytosis and/or neuronal death, methods of treating an A β -related disease or disorder, and methods of treating a disease or disorder associated with neuroinflammation using a therapeutically effective amount of an anti-CD22 (*e.g.*, human CD22) antibody or antigen-binding fragment disclosed herein, such as SM03, SM06 or their variants or equivalents. Without being bound by theory, it is to be understood that the SM03, SM06, and other anti-CD22 antibodies disclosed herein can be used to achieve the above-mentioned therapeutic effect because they have at least one of the following functions or activities: (1) binding to an epitope that does not sterically interfere CD22 interaction with A β ; (2) binding to the domain 2 of human CD22; (3) binding to the conformational epitope of human CD22 encompassing the sequence $^{161}\text{CLLNFSYGYPIQ}_{173}$ (SEQ ID NO:17) and $^{198}\text{VFTRSELKFSPQWSHHGKIVTC}_{219}$ (SEQ ID

NO:18); (4) disrupting *cis*-binding of CD22 homo-cluster; (5) promoting the *cis-trans* conversion of 2,6-sialic acid binding of CD22; (6) promoting *trans*-binding of CD22 to neuronal 2,6-sialic acid; (7) promoting CD22 internalization on, *e.g.*, microglia cells, Ramos cell, and/or B cell, optionally at a rate of 50% of the surface CD22 internalized within 10 minutes; (8) promoting internalization of A β in microglia cells; (9) suppressing pro-inflammatory cytokines, such as NF κ B signaling, and IL-6 secretion; and (10) reducing neuroinflammation.

[00163] It is to be understood that antibodies or antigen-binding fragments that specifically bind domain 2 of human CD22, and/or the conformational epitope of human CD22 encompassing the sequence ₁₆₁CLLNFSYGYPIQ₁₇₃ (SEQ ID NO:17) and ₁₉₈VFTRSELKFSPQWSHHGKIVTC₂₁₉ (SEQ ID NO:18) can promote the *cis-trans* conversion of neuronal 2,6-sialic acid binding of CD22, thereby inducing rapid internalization of CD22 on microglia cells. The rapid internalization of CD22 then leads to rapid internalization of both CD22-bound A β and soluble A β by microglia cells without engaging Fc γ R on the cell surface, thereby avoiding ARIA-E and ARIA-H that result from the Fc γ R involvement. Additionally, both the internalization of CD22 and the removal of A β can suppress inflammatory cytokines, such as NF κ B signaling, and IL-6 secretion, thereby reducing neuroinflammation.

[00164] As such, provided herein are also methods for selecting for antibodies or antigen-binding fragments that specifically bind to CD22 (*e.g.*, human CD22), for at least one of the following functions or activities, wherein the selected anti-CD22 antibody or antigen-binding fragment can be used in methods of promoting the removal of A β plaque, methods of reducing neuroinflammation, methods of preventing synaptic phagocytosis and/or neuronal death, methods of treating an A β -related disease or disorder, and/or methods of treating a disease or disorder associated with neuroinflammation: (1) binding to an epitope that does not sterically interfere CD22 interaction with A β ; (2) binding to the domain 2 of human CD22; (3) binding to the conformational epitope of human CD22 encompassing the sequence ₁₆₁CLLNFSYGYPIQ₁₇₃ (SEQ ID NO:17) and ₁₉₈VFTRSELKFSPQWSHHGKIVTC₂₁₉ (SEQ ID NO:18); (4) disrupting *cis*-binding of CD22 homo-cluster; (5) promoting the *cis-trans* conversion of 2,6-sialic acid binding of CD22; (6) promoting *trans*-binding of CD22 to neuronal 2,6-sialic acid; (7) promoting CD22 internalization on, *e.g.*, microglia cells, Ramos cell, and/or B cell, optionally at a rate of 50% CD22 internalized by 10 minutes; (8) promoting internalization of A β in microglia cells, optionally at a rate of 5.86×10^{-6} pg/s/cell; (9) reducing neuroinflammation; and (10) suppressing pro-inflammatory cytokines, such as NF κ B signaling, and IL-6 secretion. In some embodiments, the selected antibodies or antigen-binding fragments can be used in methods of promoting the removal of A β plaque. In some

embodiments, the selected antibodies or antigen-binding fragments can be used in methods of reducing neuroinflammation. In some embodiments, the selected antibodies or antigen-binding fragments can be used in methods of preventing synaptic phagocytosis and/or neuronal death. In some embodiments, the selected antibodies or antigen-binding fragments can be used in methods of treating an A β -related disease or disorder. In some embodiments, the selected antibodies or antigen-binding fragments can be used in methods of treating a disease or disorder associated with neuroinflammation.

[00165] For illustrative purposes, epratuzumab (Emab) was identified to be capable of inducing CD22 internalization and confirmed to also promote A β removal in microglia cells. Accordingly, Emab and its functional variants can be used in methods disclosed herein, including methods of promoting the removal of A β plaque, methods of reducing neuroinflammation, methods of preventing synaptic phagocytosis and/or neuronal death, methods of treating an A β -related disease or disorder, and/or methods of treating a disease or disorder associated with neuroinflammation.

[00166] In some embodiments, the anti-CD22 antibody or antigen-binding fragment to be used in the methods disclosed herein is selected for specifically binding to the domain 2 of human CD22 or the conformational epitope of human CD22 encompassing the sequence ₁₆₁CLLNFSYGYPIQ₁₇₃ (SEQ ID NO:17) and ₁₉₈VFTRSELKFSPQWSHHGKIVTC₂₁₉ (SEQ ID NO:18). In some embodiments, the anti-CD22 antibody or antigen-binding fragment to be used in the methods disclosed herein is selected for its binding to LRID (*e.g.*, US Patent No. US 9,371,396 B2).

[00167] It was found by the inventors of the current disclosure that amongst different anti-human CD22 antibodies, the closer its binding epitope is to the cell membrane, the slower the rate of internalization that the antibody induces. Accordingly, by epitope-based selection, namely, selecting for anti-CD22 antibodies that bind to an epitope distant from the cell membrane, antibodies that induce rapid internalization can be identified. Any method of epitope mapping that is disclosed herein or otherwise known in the art can be used, such as mutagenesis, including but not limited to, shotgun mutagenesis, site-directed mutagenesis, and alanine scanning; domain or fragment scanning; peptide scanning (*e.g.*, Pepscan technology); display methods (*e.g.*, phage display, microbial display, and ribosome/mRNA display); methods involving proteolysis and mass spectroscopy; and structural determination (*e.g.*, X-ray crystallography and NMR).

[00168] In some embodiments, antibodies or antigen-binding fragments thereof that specifically bind to discontinuous conformation epitope in domain 2 induce rapid internalization as disclosed herein can be isolated by screening of a recombinant combinatorial antibody library, preferably a scFv phage display library, prepared using human VL and VH cDNAs prepared from mRNA derived from

human lymphocytes. Methodologies for preparing and screening such libraries are known in the art. In addition to commercially available kits for generating phage display libraries (*e.g.*, the GE Healthcare Life Sciences Recombinant Antibody Phage System (RAPS); and the New England Biolab Ph.DTM Phage Display Library Kit, catalog no. E8100S), examples of methods and reagents particularly amenable for use in generating and screening antibody display libraries can be found in, for example, Ladner *et al.* U.S. Pat. No. 5,223,409; Fuchs *et al.* (1991) *Biotechnology* 9:1369-1372; Hay *et al.* (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989) *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.* (1992) *J Mol Biol* 226:889-896; Clackson *et al.* (1991) *Nature* 352:624-628; Gram *et al.* (1992) *PNAS* 89:3579-3580; Garrard *et al.* (1991) *Biotechnology* 9:1373-1377; Hoogenboom *et al.* (1991) *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991) *PNAS* 88:7978-7982.

[00169] In some embodiments, the anti-CD22 antibody or antigen-binding fragment to be used in the methods disclosed herein is further selected for binding to human CD22 with a K_a of $0.8 \times 10^9 \text{ M}^{-1}$ or higher. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for binding to human CD22 with a K_D of about 1.2 nM or lower. In some embodiments, the anti-CD22 antibody or antigen-binding fragment to be used in the methods disclosed herein is selected for dissociating from human CD22 with a k_d of 0.07 RU s^{-1} or less, or a k_d of 0.015 RU s^{-1} or less. The binding parameters mentioned herein can be determined by any methods disclosed herein or known in the art, for example, by surface plasmon resonance. In some embodiments, the anti-CD22 antibody or antigen-binding fragment to be used in the methods disclosed herein is further selected for its function of inducing CMC activities against a surrogate target cell expressing surface binding moieties of the LRID with an EC_{50} of $0.2 \mu\text{g/ml}$ or less.

[00170] In some embodiments, to isolate murine, recombinant or human antibodies with high affinity and a low off rate constant for human CD22, a parent murine or chimeric anti-CD22 antibody having high affinity and a low off rate constant for human CD22 (*e.g.*, the hybridoma for murine anti-CD22 antibody deposited in ATCC with accession number: 7621) is first used to select human heavy and light chain sequences having similar binding activity toward human CD22, using the epitope imprinting methods described in PCT Publication No. WO 93/06213. The antibody libraries used in this method are preferably scFv libraries prepared and screened as described in PCT Publication No. WO 92/01047, McCafferty *et al.*, *Nature* (1990) 348:552-554; and Griffiths *et al.*, (1993) *EMBO J* 12:725-734. The scFv antibody libraries preferably are screened using recombinant human CD22 (2-

4 domains), and/or an anti-idiotypic antibody specific for the antigen binding site (ABS) of the anti-CD22 antibody (*e.g.*, LRID as described in US Pat. No. 9371396B) as the antigen.

[00171] Once initial human VL and VH segments are selected, “mix and match” experiments, in which different pairs of the initially selected VL and VH segments are screened for human CD22 binding, are performed to select preferred VL/VH pair combinations. The affinity and/or specificity of binding of an antibody for a particular antigen can be increased using methods of “directed evolution,” as described by Wu et al., *J. Mol. Biol.*, 294:151 (1999), the contents of which are incorporated herein by reference herein in their entirety. Additionally, to further improve the affinity and/or lower the off-rate constant for human CD22 (or recombinant CD22 (2-4) domains) binding, the VL and VH segments of the preferred VL/VH pair(s) can be randomly mutated, preferably within the CDR3 region of VH and/or VL, in a process analogous to the *in vivo* somatic mutation process responsible for affinity maturation of antibodies during a natural immune response. This *in vitro* affinity maturation can be accomplished by amplifying VH and VL regions using PCR primers complementary to the VH CDR3 or VL CDR3, respectively, which primers have been “spiked” with a random mixture of the four nucleotide bases at certain positions such that the resultant PCR products encode VH and VL segments into which random mutations have been introduced into the VH and/or VL CDR3 regions. These randomly mutated VH and VL segments can be rescreened for binding to human CD22, preferably to the recombinant protein containing domains 2-4 of CD22, and/or binding to an anti-idiotypic antibody of the anti-CD22 antibody (*e.g.*, LRID); and sequences that exhibit high affinity and a low off rate for human CD22, preferably recombinant CD22 (2-4) domain, and/or an anti-idiotypic antibody for CD22 (*e.g.*, LRID) binding can be selected.

[00172] In addition to phage display, candidate monoclonal antibodies for screening can also be prepared using hybridoma methods, which are well known to one of skill in the art. For example, using a hybridoma method, a mouse, rat, rabbit, hamster, or other appropriate host animal, is immunized as described above. In some embodiments, lymphocytes are immunized *in vitro*. In some embodiments, the immunizing antigen is a human protein or a fragment thereof. In some embodiments, the immunizing antigen is a human protein or a fragment thereof.

[00173] Following immunization, lymphocytes are isolated and fused with a suitable myeloma cell line using, for example, polyethylene glycol. The hybridoma cells are selected using specialized media as known in the art and unfused lymphocytes and myeloma cells do not survive the selection process. Hybridomas that produce monoclonal antibodies directed to a chosen antigen can be identified by a variety of methods including, but not limited to, immunoprecipitation, immunoblotting, and *in vitro* binding assays (*e.g.*, flow cytometry, FACS, ELISA, BLI, SPR (*e.g.*,

Biacore), and radioimmunoassay). Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution or other techniques. The hybridomas can be propagated either in *in vitro* culture using standard methods or *in vivo* as ascites tumors in an animal. The monoclonal antibodies can be purified from the culture medium or ascites fluid according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

[00174] Additional methods of screening for an antibody or antigen-binding fragment that is disruptive of CD22 binding in *cis* have been previously disclosed by the inventors of the present disclosure (*e.g.*, WO2020078453A1, incorporated herein by reference in its entirety). In some embodiments, the screening methods comprise using an engineered cell line expressing a fusion protein comprising non-internalizing, human CD22 domain 1 to 7 and a domain that contains the 2,6-sialic acid ligand, by comparing the binding of an exogenous probe containing multiple 2,6-sialic acids to the engineered cell line in the presence and absence the antibody, wherein the antibody is identified as capable of disrupting CD22 *cis*-binding if it restores the binding the exogenous probe to the engineered cell line.

[00175] In some embodiments, the fusion protein comprises human CD22 domain 1 to 7 and glycoporphin A. In some embodiments, the fusion protein comprises the extracellular domain of human CD22 fused to the transmembrane and cytoplasmic portion of glycoporphin A. In some embodiments, the fusion protein comprises the extracellular domain of human CD22 fused to the glycoposphatidylinositol signal sequence isolated from decay accelerating factor (DAF) protein.

[00176] In some embodiments, wherein the fusion protein has an amino acid sequence comprising that is at least 95% identical to SEQ ID NO:15. In another embodiment, the fusion protein has an amino acid sequence being at least 95, 96, 97, 98 or 99% identical to SEQ ID NO: 15. In another embodiment, the fusion protein has the amino acid sequence of SEQ ID NO:15. In some embodiments, wherein the fusion protein has an amino acid sequence comprising that is at least 95% identical to SEQ ID NO:16. In another embodiment, the fusion protein has an amino acid sequence being at least 95, 96, 97, 98 or 99% identical to SEQ ID NO: 15. In another embodiment, the fusion protein has the amino acid sequence of SEQ ID NO:16.

[00177] In some embodiments, the exogenous probe is biotin-conjugated polyacrylamide substituted with α 2-6-sialyllactose (6'PAA-B; Glycotech).

[00178] In some embodiments, a non-human antibody identified to have the therapeutic uses disclosed herein via the screening methods disclosed herein can be humanized, where specific

sequences or regions of the antibody are modified to increase similarity to an antibody naturally produced in a human. In some embodiment, the antigen binding domain portion is humanized.

[00179] Often, framework residues in the framework regions can be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well-known in the art, *e.g.*, by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (*See, e.g.*, Queen *et al.*, U.S. Pat. No. 5,585,089; and Riechmann *et al.*, 1988, *Nature*, 332:323, which are incorporated herein by reference in their entireties.)

[00180] A humanized antibody has one or more amino acid residues introduced into it from a source which is nonhuman. These nonhuman amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Thus, humanized antibodies comprise one or more CDRs from nonhuman immunoglobulin molecules and framework regions from human. Methods of humanization of antibodies are well-known in the art, including CDR-grafting (*see, e.g.*, Jones *et al.*, *Nature*, 321:522-525 (1986); Riechmann *et al.*, *Nature*, 332:323-327 (1988); Verhoeven *et al.*, *Science*, 239:1534-1536 (1988); European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 4,816,567; 6,331,415; 5,225,539; 5,530,101; 5,585,089; and 6,548,640; each of which is incorporated herein in its entirety by reference). In such humanized antibodies, substantially less than an intact human variable domain has been substituted by the corresponding sequence from a nonhuman species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies. Methods of producing humanized antibodies also include veneering or resurfacing (*see, e.g.*, European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, *Molecular Immunology*, 28(4/5):489-498; Studnicka *et al.*, 1994, *Protein Engineering*, 7(6):805-814; and Roguska *et al.*, 1994, *PNAS*, 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (*see, e.g.*, U.S. Pat. No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, *e.g.*, U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan *et al.*, *J. Immunol.*, 169:1119-25 (2002), Caldas *et al.*, *Protein Eng.*, 13(5):353-60 (2000), Morea *et al.*, *Methods*, 20(3):267-79 (2000), Baca *et al.*, *J. Biol. Chem.*, 272(16):10678-84 (1997), Roguska *et al.*, *Protein Eng.*, 9(10):895-904 (1996), Couto *et al.*, *Cancer Res.*, 55 (23 Supp):5973s-5977s (1995), Couto *et al.*, *Cancer Res.*, 55(8):1717-22 (1995), Sandhu J S, *Gene*, 150(2):409-10 (1994), and

Pedersen *et al.*, *J. Mol. Biol.*, 235(3):959-73 (1994), each of which is incorporated herein in its entirety by reference.

[00181] The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is to reduce antigenicity. According to the so-called “best-fit” method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized antibody (Sims *et al.*, *J. Immunol.*, 151:2296 (1993); Chothia *et al.*, *J. Mol. Biol.*, 196:901 (1987), the contents of which are incorporated herein by reference herein in their entirety). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta *et al.*, *J. Immunol.*, 151:2623 (1993), the contents of which are incorporated herein by reference herein in their entirety).

[00182] Antibodies can be humanized with retention of high affinity for the target antigen and other favorable biological properties. For example, humanized antibodies can be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind the target antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen, is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[00183] Following screening and isolation of an anti-CD22 antibody disclosed herein from a recombinant immunoglobulin display library, nucleic acid encoding the selected antibody can be recovered from the display package (*e.g.*, from the phage genome) and subcloned into other expression vectors by standard recombinant DNA techniques. If desired, the nucleic acid can be further manipulated to create other antibody forms disclosed herein or known in the art (*e.g.*, linked to a nucleic acid encoding additional immunoglobulin domains, such as additional constant regions). Methods to express a recombinant human antibody isolated by screening of a combinatorial library,

including cloning the DNA encoding the antibody into a recombinant expression vector and introduced into a mammalian host cell, are well known in the art.

[00184] In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for (1) binding to an epitope that does not sterically interfere CD22 interaction with A β , and (2) disrupting *cis*-binding of CD22 homo-cluster, promoting the *cis-trans* conversion of 2,6-sialic acid binding of CD22, and/or promoting *trans*-binding of CD22 to neuronal 2,6-sialic acid. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is directly selected for its function of promoting CD22 internalization, promoting internalization of A β in microglia cells, preferably without engaging Fc γ R on the cell surface, reducing neuroinflammation, suppressing NF κ B signaling in microglia cells, suppressing IL-6 secretion by microglia cells, or any combination thereof.

[00185] In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for its function of promoting CD22 internalization. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for its function of promoting internalization of A β in microglia cells, preferably without engaging Fc γ R on the cell surface. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for its function of reducing neuroinflammation. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for its function of suppressing NF κ B signaling in microglia cells. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for its function of suppressing IL-6 secretion by microglia cells. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for its function of suppressing neuroinflammation. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for its function of suppressing synaptic phagocytosis. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is selected for its function of suppressing neuronal death.

[00186] Anti-CD22 antibodies or antigen-binding fragments described herein can be tested for binding to human CD22 by, for example, standard ELISA. Briefly, microtiter plates are coated with purified CD22, and then blocked with bovine serum albumin. Dilutions of antibody (*e.g.*, dilutions of plasma from CD22-immunized mice) are added to each well and incubated. The plates are washed and incubated with secondary reagent (*e.g.*, for human antibodies, a goat-anti-human IgG Fc-specific polyclonal reagent) conjugated to horseradish peroxidase (HRP). After washing, the plates can be developed and analyzed by a spectrophotometer. Sera from immunized mice can then be further screened by flow cytometry for binding to a cell line expressing human CD22, but not to a control cell line that does not express CD22. Briefly, the binding of anti-CD22 antibodies can be assessed by incubating CD22 expressing CHO cells with the anti-CD22 antibody. The cells can be washed and

binding can be detected with an anti-human IgG Ab. Flow cytometric analyses can be performed using a FACScan flow cytometry (Becton Dickinson, San Jose, CA). Mice which develop the highest titers can be used for fusions.

[00187] An ELISA assay as described above can be used to screen for antibodies and, thus, hybridomas that produce antibodies that show positive reactivity with the CD22 immunogen. Hybridomas that produce antibodies that bind with high affinity to CD22 can then be subcloned and further characterized. One clone from each hybridoma, which retains the reactivity of the parent cells (by ELISA), can then be chosen for making a cell bank, and for antibody purification.

[00188] To purify anti-CD22 antibodies, selected hybridomas can be grown for monoclonal antibody purification. Supernatants can be filtered and concentrated before affinity chromatography. Eluted IgG can be checked by gel electrophoresis and high-performance liquid chromatography to ensure purity. The buffer solution can be exchanged, and the concentration can be determined. The monoclonal antibodies can be aliquoted and stored.

[00189] A variety of methods and assays are known in the art to determine the structural and functional properties of the antibodies and antigen-binding fragments. For example, to determine if the selected anti-CD22 monoclonal antibodies bind to unique epitopes, each antibody can be biotinylated using commercially available reagents (Pierce, Rockford, IL). Biotinylated MAb binding can be detected with a streptavidin labeled probe. Competition studies using unlabeled monoclonal antibodies and biotinylated monoclonal antibodies can be performed using CD22 coated-ELISA plates.

[00190] To test the binding of monoclonal antibodies to live cells expressing CD22, flow cytometry can be used. Briefly, CD22-expressing cell lines (grown under standard growth conditions) are mixed with various concentrations of monoclonal antibodies in PBS containing 0.1% BSA at 4 °C for 1 hour. After washing, the cells are reacted with Fluorescein-labeled anti-IgG antibody under the same conditions as the primary antibody staining. The samples can be analyzed by FACScan instrument using light and side scatter properties to gate on single cells and binding of the labeled antibodies is determined. An alternative assay using fluorescence microscopy can be used (in addition to or instead of) the flow cytometry assay. Cells can be stained exactly as described above and examined by fluorescence microscopy. This method allows visualization of individual cells, but can have diminished sensitivity depending on the density of the antigen.

[00191] Methods for analyzing binding affinity, cross-reactivity, and binding kinetics of various anti-CD22 antibodies include standard assays known in the art, for example, biolayer interferometry (BLI) using, for example, Gator system (Probe Life) or the Octet-96 system (Sartorius AG), or

BIACORE™ surface plasmon resonance (SPR) analysis using a BIACORE™ 2000 SPR instrument (Biacore AB, Uppsala, Sweden).

[00192] Assays to test or screen anti-CD22 antibodies or antigen-binding fragments for functional properties disclosed above are well known in the art. Any methods disclosed herein or otherwise known in the art can be used in the methods of screening disclosed herein. For example, the functional assays that can be used in the screening methods disclosed herein include the luciferase assay for measuring NFκB signaling, the ELISA assay for measuring IL-6 release, the ELISA or Western Blot assay for measuring synaptic phagocytosis, and the MTT assay for measuring neuronal death, all of which are disclosed in the experimental section below.

[00193] In some embodiments, provided herein are also the anti-CD22 antibodies and antigen-binding fragments identified or produced using the methods described herein, as well as their therapeutic uses.

5.3 Methods of Production

[00194] The anti-CD22 antibodies and antigen-binding fragments thereof that that can be used in methods disclosed herein, including but not limited to monoclonal antibodies, chimeric antibodies, human antibodies, and humanized antibodies, can be prepared by any methods disclosed herein or otherwise known in the art.

[00195] Methods of antibody production are well-known in the art. See for example, in Harlow *et al.*, ANTIBODIES: A LABORATORY MANUAL, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: Monoclonal Antibodies and T-Cell Hybridomas 563, 681 (Elsevier, N.Y., 1981), each of which is incorporated herein in its entirety by reference.

[00196] In some embodiments, the antibodies or antigen-binding fragments that can be used in methods provided herein are recombinant, namely, prepared, expressed, produced or isolated by recombinant means. In some embodiments, the antibodies or antigen-binding fragments disclosed herein can be prepared, for example, by introducing recombinant expression vectors into host cells, a recombinant, combinatorial human antibody library, antibodies isolated from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (*see e.g.*, Taylor, L.D., *et al.* (1992) *Nucl. Acid Res.* 20:6287-95) or antibodies prepared, expressed, produced, or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences.

[00197] In some embodiments, antibodies and antigen-binding fragments can be prepared by recombinant expression of immunoglobulin light and heavy chain genes in a host cell. To express an antibody recombinantly, a host cell is introduced with one or more recombinant expression vectors carrying DNA fragments encoding the immunoglobulin light and heavy chains of the antibody such

that the light and heavy chains are expressed in the host cell and, preferably, secreted into the medium in which the host cells are cultured, from which medium the antibodies can be recovered. Standard recombinant DNA methodologies are used to obtain antibody heavy and light chain genes, incorporate these genes into recombinant expression vectors and introduce the vectors into host cells, such as those described in Sambrook, Fritsch and Maniais (eds), *MOLECULAR CLONING: A LABORATORY MANUAL*, Second Edition, Cold Spring Harbor, N.Y., (1989), Ausubel *et al.* (eds.) *CURRENT PROTOCOLS IN MOLECULAR BIOLOGY*, Greene Publishing Associates, (1989) and in U.S. Pat. No. 4,816,397.

[00198] To express a recombinant antibody or antigen fragment, such as SM03, SM06 or a SM03/SM06-related antibody, DNA fragments encoding the light and heavy chain variable regions are first obtained. These DNAs can be obtained by amplification and modification of hybridomas for the murine antibody light and heavy chain variable sequences using the polymerase chain reaction (PCR), or by oligosynthesis based on the encoded amino acid sequence of design light and heavy chain variable sequences using standard methods known to those skilled in the art. The encoding DNA sequences can be further optimized to facilitate mammalian expression of the resultant antibody.

[00199] Once the VH and VL fragments for the murine antibody are obtained, these sequences can be mutated to encode the framework-patched version of SM03 (*i.e.*, SM06), the method of which was described in Chinese Pat. Nos. 01144894.6 and 031 23054.7 and US Pat. No. 7,321,026 B2 & 7,338,659 B2, both incorporated herein in their entirety by reference.

[00200] Once DNA fragments encoding SM03 or SM06, or SM03/SM06-related VH and VL segments are obtained (by, *e.g.*, amplification and mutagenesis of the original murine VH and VL genes, as described above), these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term “operatively linked,” as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[00201] The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., et al (1991) *SEQUENCES OF PROTEINS OF IMMUNOLOGICAL*

INTEREST, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, Ig4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

[00202] The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., et al (1991) SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a kappa constant region.

[00203] To prepare a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence (Gly4-Ser)3, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.* (1990) *Nature* 348:552-554).

[00204] To express the antibodies, or antigen-binding fragments that can be used in the methods disclosed herein, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term “operatively linked” is intended to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). In some embodiments, prior to insertion of the SM03, SM06 or SM03/SM06-related light or heavy chain

sequences, the expression vector already carries antibody constant regions sequences. For example, one approach to convert the SM03 or SM06 or SM03/SM06-related VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

[00205] In addition to the antibody chain genes, the recombinant expression vectors provided herein can carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term “regulatory sequence” is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). As those skilled in the art would appreciate, the design of the expression vector, including the selection of regulatory sequences depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from immunoglobulin heavy chain (IgH) enhancer (Gillies et al. (1983) Cell 33:717-728) metallothioneine (MT), cytomegalovirus (CMV)(such as the CMV promoter/enhancer), Simian Virus 40 (SV40)(such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, *see e.g.*, U.S. Pat. Nos. 5,665,578; 5,168,062; 4,510,245; and 4,968,615.

[00206] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors provided herein can carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U. S. Pat. Nos. 4,399,216, 4,634,665 and 5,179,017). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the

dihydrofolate reductase (DHFR) gene (for use in dhfr- host cells with methotrexate selection/amplification), Glutamate Synthase (GS) gene and the neo gene (for G418 selection).

[00207] For expression of the light and heavy chains, the expression vector encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term “transfection” are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection, lipofection, protoplast fusion and the like. Although antibodies and antigen-binding fragments can be produced in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, especially mammalian host cells, is preferred because such host cells are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

[00208] Preferred mammalian host cells for expressing the recombinant antibodies used in methods described herein include SP2/0 myeloma cells, NSO myeloma cells, COS cells, and Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4200, used with a DHFR selectable marker, *e.g.*, as described in R. J. Kaufman and P. A. Sharp (1982) *J. Mol. Biol.* 159:601-621). When recombinant antibody-encoding expression vectors are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

[00209] Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. Expressly contemplated herein are variations of the above procedure. For example, it can be desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody used in methods disclosed herein. Recombinant DNA technology can also be used to remove some or all of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to CD22. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies provided herein. In addition, bifunctional antibodies can be produced in which one heavy and one light chain are an antibody that specifically binds human CD22, and the other heavy and light chain are specific for an antigen other than CD22 by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

[00210] In some embodiments of the systems of recombinant expression of an antibody or antigen-binding fragment thereof that can be used in the methods disclosed herein, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into SP2/0 cells by electroporation. In some embodiments of the expression systems, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into CHO cells by standard techniques such as lipofection.

[00211] Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to murine or human Immunoglobulin heavy chain (IgH), CMV enhancer, metallothioneine or AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of SP2/0 cells that have been transfected with the vector using methotrexate selection/amplification. Alternatively, the recombinant expression vector containing the antibody heavy and light chain genes operatively linked to murine or human IgH, CMV enhancer/AdMLP/metallothioneine promoter regulatory elements and a DHFR gene can be used to transfect SP2/0 or CHO cells that are dhfr-. SP2/0 or CHO cells transfected with the vector can be selected and the level of gene expression in the vector amplified by increasing the levels of methotrexate in the culture. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium.

5.4 Pharmaceutical Compositions

[00212] Provided herein are also pharmaceutical compositions comprising the anti-CD22 antibodies or antigen-binding fragments (*e.g.*, SM03, SM06) that can be used in methods disclosed herein. In some embodiments, the pharmaceutical composition comprises a therapeutically effective amount of the anti-CD22 antibodies or antigen-binding fragments disclosed herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical compositions are useful in the treatment of an A β -related disease or disorder or a neuroinflammation-related disease or disorder. In some embodiments, the pharmaceutical compositions are useful in treating AD. In some embodiments, the pharmaceutical compositions are useful in inhibiting AD progression in a subject (*e.g.*, a human patient). In some embodiments, the pharmaceutical compositions are useful in ameliorating cognitive impairment in a subject (*e.g.*, a human patient).

[00213] The amount of therapeutic antibody which can be combined with a carrier material in the pharmaceutical compositions disclosed herein can vary. In some embodiments, the amount of

antibodies present in the pharmaceutical compositions is the amount that produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 0.01 percent to about ninety-nine percent of active ingredient, from about 0.1 percent to about 70 percent, or from about 1 percent to about 30 percent of active ingredient in combination with a pharmaceutically acceptable carrier.

[00214] The pharmaceutical compositions provided herein comprise anti-CD22 antibodies or antigen-binding fragments provided herein, *e.g.*, SM03, SM06, or SM03/SM06-related antibodies, or identified by the methods disclosed herein. The anti-CD22 antibodies or antigen-binding fragments can be present at various concentrations. In some embodiments, the pharmaceutical compositions provided herein comprise soluble anti-CD22 antibodies or antigen-binding fragments provided herein at 1-1000 mg/ml. In some embodiments, the pharmaceutical compositions comprise soluble anti-CD22 antibodies or antigen-binding fragments provided herein at 10-500 mg/ml, 10-400 mg/ml, 10-300 mg/ml, 10-200 mg/ml, 10-100 mg/ml, 20-100 mg/ml, or 50-100 mg/ml. In some embodiments, the pharmaceutical compositions provided herein comprise anti-CD22 antibodies or antigen-binding fragments provided herein at about 10 mg/ml, about 20 mg/ml, about 30 mg/ml, about 40 mg/ml, about 50 mg/ml, about 60 mg/ml, about 70 mg/ml, about 80 mg/ml, about 90 mg/ml, about 100 mg/ml, about 120 mg/ml, about 150 mg/ml, about 180 mg/ml, about 200 mg/ml, about 300 mg/ml, about 500 mg/ml, about 800 mg/ml, or about 1000 mg/ml. Dosages can be readily adjusted by those skilled in the art; for example, a decrease in purity requires an increase in dosage.

[00215] The pharmaceutical compositions provided herein can be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Examples of suitable aqueous and nonaqueous carriers that can be employed in the pharmaceutical compositions or formulations described herein include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate.

[00216] Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. In some embodiments, pharmaceutical compositions provided herein are in the form of injectable or infusible solutions. In some embodiments, the pharmaceutical composition is an aqueous formulation. Such a formulation is typically a solution or a suspension, but can also include colloids, dispersions, emulsions, and multi-phase materials. The term “aqueous formulation” is

defined as a formulation comprising at least 50% w/w water. Likewise, the term “aqueous solution” is defined as a solution comprising at least 50 % w/w water, and the term “aqueous suspension” is defined as a suspension comprising at least 50 % w/w water. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration.

[00217] In some embodiments, the pharmaceutical compositions disclosed herein are freeze-dried, to which the physician or the patient adds solvents and/or diluents prior to use.

[00218] The pharmaceutical compositions provided herein can comprise a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In some embodiments, the pharmaceutical acceptable carriers include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition.

[00219] In some embodiments, the pharmaceutical acceptable carriers further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antigen-binding fragment. In some embodiments, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (*e.g.*, by injection or infusion). Depending on the route of administration, the active ingredient (*i.e.*, anti-CD22 antibodies or antigen-binding fragments), can be coated in a material to protect the active ingredient from the action of acids and other natural conditions that can inactivate the active ingredient.

[00220] Provided herein are also kits for preparation of pharmaceutical compositions having the anti-CD22 antibodies or antigen-binding fragments disclosed herein, *e.g.*, SM03, SM06, or SM03/SM06-related antibodies. In some embodiments, the kit comprises the anti-CD22 antibodies or antigen-binding fragments disclosed herein and a pharmaceutically acceptable carrier in one or more containers. In another embodiment, the kits can comprise anti-CD22 antibodies or antigen-binding fragments disclosed herein for administration to a subject. In specific embodiments, the kits comprise instructions regarding the preparation and/or administration of the anti-CD22 antibodies or antigen-binding fragments.

[00221] In some embodiments, the pharmaceutical composition or formulation disclosed herein comprises: (a) anti-CD22 antibodies or antigen-binding fragments disclosed herein; (b) a buffering agent; (c) a stabilizing agent; (d) a salt; (e) a bulking agent; and/or (f) a surfactant. In some embodiments, the pharmaceutical composition or formulation is stable for at least 1 month, at least 2

months, at least 3 months, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 5 years or more. In some embodiments, the pharmaceutical composition or formulation is stable when stored at 4°C, 25°C, or 40°C. In some embodiments, provided herein are also pharmaceutical compositions or formulations that improve the stability of the anti-CD22 antibodies or antigen-binding fragments to allow for their long-term storage. The pharmaceutical compositions disclosed herein can further comprise one or more of a preservative, a tonicity agent, a chelating agent, a stabilizer and/or a surfactant, as well as various combinations thereof. The use of preservatives, isotonic agents, chelating agents, stabilizers and surfactants in pharmaceutical compositions is well-known to the skilled person. Reference may be made to Remington: THE SCIENCE AND PRACTICE OF PHARMACY, 19th edition, 1995.

[00222] Buffering agents useful in the pharmaceutical compositions or formulations disclosed herein can be a weak acid or base used to maintain the acidity (pH) of a solution near a chosen value after the addition of another acid or base. Suitable buffering agents can maximize the stability of the pharmaceutical formulations by maintaining pH control of the formulation. Suitable buffering agents can also ensure physiological compatibility or optimize solubility. Rheology, viscosity and other properties can also depend on the pH of the formulation. Common buffering agents include, but are not limited to, histidine, citrate, succinate, acetate and phosphate. In some embodiments, a buffering agent comprises histidine (*e.g.*, L-histidine) with isotonicity agents and potentially pH adjustment with an acid or a base known in the art. In certain embodiments, the buffering agent is L-histidine. In certain embodiments, the pH of the formulation is maintained between about 2 and about 10, or between about 4 and about 8.

[00223] Stabilizing agents are added to a pharmaceutical product to stabilize that product. Such agents can stabilize proteins in different ways. Common stabilizing agents include, but are not limited to, amino acids such as glycine, alanine, lysine, arginine, or threonine, carbohydrates such as glucose, sucrose, trehalose, raffinose, or maltose, polyols such as glycerol, mannitol, sorbitol, cyclodextrins or destrans of any kind and molecular weight, or PEG. In some embodiments, the stabilizing agent is chosen to maximize the stability of FIX polypeptide in lyophilized preparations. In certain embodiments, the stabilizing agent is sucrose and/or arginine.

[00224] Bulking agents can be added to a pharmaceutical composition or formulation to add volume and mass to the product, thereby facilitating precise metering and handling thereof. Common bulking agents include, but are not limited to, lactose, sucrose, glucose, mannitol, sorbitol, calcium carbonate, or magnesium stearate.

[00225] Surfactants are amphipathic substances with lyophilic and lyophobic groups. A surfactant can be anionic, cationic, zwitterionic, or nonionic. Examples of nonionic surfactants include, but are not limited to, alkyl ethoxylate, nonylphenol ethoxylate, amine ethoxylate, polyethylene oxide, polypropylene oxide, fatty alcohols such as cetyl alcohol or oleyl alcohol, cocamide MEA, cocamide DEA, polysorbates, or dodecyl dimethylamine oxide. In some embodiments, the surfactant is polysorbate 20 or polysorbate 80.

[00226] Pharmaceutical compositions disclosed herein can also include a pharmaceutically acceptable antioxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[00227] These compositions can also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms can be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It can also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form can be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[00228] Pharmaceutical compositions or formulations typically must be sterile and stable under the conditions of manufacture and storage. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Sterile injectable solutions can be prepared by incorporating the therapeutic antibody or antigen-binding fragment in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. The use of such media and agents for pharmaceutically active substances is known in the art. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, some methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00229] The pharmaceutical compositions disclosed herein can be prepared with carriers that protect the active ingredient against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and poly lactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g.*, SUSTAINED AND CONTROLLED RELEASE DRUG DELIVERY SYSTEMS, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[00230] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments described herein can be formulated to ensure proper distribution *in vivo*. For example, the BBB excludes many highly hydrophilic compounds. To facilitate the therapeutic antibodies described herein to cross the BBB, they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, *see, e.g.*, U.S. Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes can comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (*see, e.g.*, V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685). Exemplary targeting moieties include folate or biotin (*see, e.g.*, U.S. Patent 5,416,016 to Low *et al*) mannosides (Umezawa *et al.*, (1988) *Biochem. Biophys. Res. Commun.* 153: 1038); antibodies (P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357: 140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39: 180); surfactant protein A receptor (Briscoe *et al.* (1995) *Am. J. Physiol.* 1233: 134); pl20 (Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090); *see also* K. Keinänen; M.L. Laukkanen (1994) *FEBS Lett.* 346: 123; J.J. Killian; I.J. Fidler (1994) *Immunomethods* 4:273.

5.5 Methods of Treatment

[00231] As described in sections above, provided herein are medical uses of anti-CD22 antibodies and antigen-binding fragments (*e.g.*, SM03 or SM06) in treating A β or neuroinflammation-related diseases or disorders. Any anti-CD22 antibody or antigen-binding fragment disclosed herein or identified using the screening methods disclosed herein can be used in the methods disclosed herein. In some embodiments, the therapeutic antibody is SM03. In some embodiments, the therapeutic antibody is SM06. In some embodiments, provided herein are methods of promoting removal of A β plaque in a subject in need thereof. In some embodiments, provided herein are also methods of treating an A β -related disease or disorder in a subject in need thereof. In some embodiments, provided herein are also methods of reducing neuroinflammation in a subject in need thereof. In some embodiments, provided herein are also methods of treating a disease or disorder associated with neuroinflammation in a subject in need thereof. In some embodiments, the methods provided

herein prevent synaptic phagocytosis and neuronal death. In some embodiments, the methods provided herein prevent synaptic phagocytosis and neuronal death by at least 20%, at least 30, at least 40%, at least over 50%, or at least over 60%. In some embodiments, the subject is a human. In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to human CD22.

[00232] The methods of promoting removal of A β plaque, reducing neuroinflammation, treating an A β -related disease or disorder, and/or treating a disease or disorder disclosed herein comprise administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes *cis-trans* conversion of CD22 and/or (b) induces internalization of CD22.

[00233] In some embodiments, the A β -related disease or disorder and/or neuroinflammation-related disease or disorder can be clinical or pre-clinical AD, prodromal AD, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma, pre-eclampsia, cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessel.

[00234] Subjects suitable for the present methods include human patients in whom the removal of A β plaque and/or reduction in neuroinflammation would be desirable. In some embodiments, the subjects to be treated with the methods disclosed herein are diagnosed with an A β -related or neuroinflammation-related disease or disorder, which can be clinical or pre-clinical AD, prodromal AD, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma, pre-eclampsia, cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessel. In some embodiments, the subjects to be treated with the methods disclosed herein are at risk of developing an A β -related or neuroinflammation-related disease or disorder, which can be clinical or pre-clinical AD, prodromal AD, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma, pre-eclampsia, cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessel. The subject can be a mammal. In some embodiments, the subject is a human.

[00235] In some embodiments, the subject is diagnosed with AD. In some embodiments, the subject is at risk of developing AD. In some embodiments, the subject has pre-clinical AD. In some embodiments, the subject has clinical AD. In some embodiments, the subject has prodromal AD. In some embodiments, the subject to be treated with the methods disclosed herein have been with the standard therapy for AD. In some embodiments, the subject has not been previously treated.

[00236] The anti-CD22 antibodies or antigen-binding fragments (*e.g.*, SM03 or SM06) or pharmaceutical compositions provided herein can be administered to a subject by any methods known in the art, including, but not limited to, intravenous administration, subcutaneous administration, intramuscular administration, intracranial administration, intrathecal administration, intraventricular administration, intraperitoneal administration, spinal administration, intranasal administration, intrapleural administration, topical administration, or intradermal administration.

[00237] In some embodiments, the anti-CD22 antibodies or antigen-binding fragments (*e.g.*, SM03 or SM06) or pharmaceutical compositions provided herein can be administered to a subject using parenteral administration. The phrase “parenteral administration” as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrastemal injection and infusion. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments are administered by intravenous infusion or injection. In some embodiments, anti-CD22 antibodies or antigen-binding fragments are administered by intramuscular injection. In some embodiments, anti-CD22 antibodies or antigen-binding fragments are administered by subcutaneous injection.

[00238] Blood brain barrier tightly regulates the substance transport in and out of the brain. As known in the art, patients with AD are associated with vascular leakage and the IgG penetration into the brain could reach approximately 0.2 %. As such, therapeutical antibodies delivered systematically can cross BBB and reach the lesion site. Alternatively, in some embodiments, the antibodies or antigen-binding fragments provided herein can be delivered locally using intracranial administration. In some embodiments, intraventricular administration is adopted.

[00239] Anti-CD22 antibodies or antigen-binding fragments (*e.g.*, SM03 or SM06) or pharmaceutical compositions provided herein can be administered with medical devices known in the art. For example, in some embodiments, a needleless hypodermic injection device can be used, such as the devices disclosed in U.S. Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules for use described herein include: U.S. Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; U.S. Patent No. 4,486,194, which discloses a therapeutic device for administering medicaments through the skin; U.S. Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; U.S. Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug

delivery; U.S. Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and U.S. Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

[00240] In some embodiments, anti-CD22 antibodies or antigen-binding fragments disclosed herein can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The therapeutic antibodies also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the therapeutic antibodies can be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like.

[00241] The methods provided herein comprise administering a therapeutically effective amount of the anti-CD22 antibodies or antigen-binding fragments (*e.g.*, SM03 or SM06) described herein. Actual dosage levels of the therapeutic antibodies can be varied so as to obtain an amount which is effective to achieve the desired therapeutic response for a particular patient, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions described herein, the route of administration, the time of administration, the rate of excretion, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[00242] Generally, the dosage can range from, *e.g.*, about 0.1 to 100 mg/kg of the host body weight for a single dose. In some embodiments, the anti-CD22 antibody or antigen-binding fragment (*e.g.*, SM03 or SM06) is administered at about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 20 mg/kg, about 30 mg/kg, about 40 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg per . In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at about 1 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at about 5 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at about 10 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at about 20 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at about 40 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at about

60 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at about 100 mg/kg.

[00243] In some embodiments, the anti-CD22 antibody or antigen-binding fragment (*e.g.*, SM03 or SM06) is administered at a dose within a range of about 1 to 5 mg/kg, about 1 to 10 mg/kg, about 1 to 20 mg/kg, about 1 to 50 mg/kg, about 1 to 100 mg/kg, about 5 to 10 mg/kg, about 5 to 20 mg/kg, about 5 to 50 mg/kg, about 5 to 100 mg/kg, about 10 to 50 mg/kg, or about 10 to 100 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at a dose within a range of about 1 to 5 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at a dose within a range of about 1 to 10 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at a dose within a range of about 1 to 50 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at a dose within a range of about 10 to 50 mg/kg. In some embodiments, the anti-CD22 antibody or antigen-binding fragment is administered at a dose within a range of about 10 to 100 mg/kg.

[00244] In some embodiments, methods provided herein comprise administering the anti-CD22 antibody or antigen-binding fragment (*e.g.*, SM03 or SM06) at a dose of about 10-2000 mg. In some embodiments, the dose is about 10 mg, about 50 mg, about 100 mg, about 200 mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1000 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, about 1800 mg, about 1900 mg, or about 2000 mg. In some embodiments, the antibody is administered at a dose of 100 mg. In some embodiments, the antibody is administered at a dose of 300 mg. In some embodiments, the antibody is administered at a dose of 600 mg. In some embodiments, the antibody is administered at a dose of 900 mg. In some embodiments, the antibody is administered at a dose of 1200 mg.

[00245] In some embodiments, methods provided herein comprise administering the anti-CD22 antibody or antigen-binding fragment (*e.g.*, SM03 or SM06) at a dose within a range of about 10-50 mg, 10-100 mg, 10-200 mg, 100-300 mg, 100-500 mg, 300-600 mg, 300-900 mg, 300-1200 mg, 600-1200 mg, 600-1800 mg, or 1000-2000 mg. In some embodiments, the antibody is administered at a dose within the range of 100-500 mg. In some embodiments, the antibody is administered at a dose within the range of 300-600 mg. In some embodiments, the antibody is administered at a dose within the range of 300-900 mg. In some embodiments, the antibody is administered at a dose within the range of 600-1200 mg.

[00246] During treatment, it is common to start with a lower dose, which is later ramped up to a target dose. For illustrative purpose, in some embodiments, the methods provided herein comprise administering an anti-CD22 antibody or antigen-binding fragment at a dose of about 100 mg, which is gradually ramped up to a target dose of about 600 mg.

[00247] Subjects can be administered at such doses daily, on alternative days, weekly, biweekly, monthly, or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. In some embodiments, the methods provided herein comprise weekly administering an anti-CD22 antibody or antigen-binding fragment. In some embodiments, the methods comprise biweekly administration. In some embodiments, the methods comprise monthly administration. In some embodiments, the anti-CD22 antibody or antigen-binding fragment (*e.g.*, SM03 or SM06) is subcutaneously administered weekly, biweekly, or monthly. In some embodiments, the anti-CD22 antibody or antigen-binding fragment (*e.g.*, SM03 or SM06) is intravenously administered weekly, biweekly, or monthly.

[00248] The anti-CD22 antibody or antigen-binding fragment (*e.g.*, SM03 or SM06) can be administered up to 3 months, 6 months, 9 months, 12 months, 18 months, 24 months, 30 months, or 36 months, as necessary and appropriate. In some embodiments, the treatment lasts at least 3 months. In some embodiments, the treatment lasts at least 6 months. In some embodiments, the treatment lasts at least 12 months. In some embodiments, the treatment lasts at least 24 months.

[00249] All permutations and combinations of the various embodiments of, *e.g.*, round of administration, dosage, treatment frequency, and length of treatment are expressly contemplated herein and can be adopted in the therapeutic methods disclosed herein.

[00250] For illustrative purposes, the following treatment regimen can be adopted in the methods disclosed herein that comprise administering of an anti-CD22 antibody or antigen binding fragment that is either disclosed herein (*e.g.*, SM03 or SM06) or identified in methods disclosed herein:

[00251] In some embodiments, the therapeutic antibody is administered intravenously or subcutaneously at a dose of about 10 mg/kg every 4 weeks and at least 21 days apart. In some embodiments, the following titration schedule is included: Infusions 1-2: 1 mg/kg IV; Infusions 3-4: 3 mg/kg IV; Infusions 5-6: 6 mg/kg IV; Infusion 7 and beyond: 10 mg/kg IV.

[00252] In some embodiments, the therapeutic antibody is administered intravenously or subcutaneously at a single dose of 10, 20, or 40 mg/kg, the second of 10 mg/kg every other week for 24 weeks, and the third of 10 or 20 mg/kg every month for 16 months.

[00253] In some embodiments, the therapeutic antibody is administered intravenously or subcutaneously at a dose of about 250 mg weekly, or 500 mg biweekly for up to 2 years. In some embodiments, the treatment starts with monthly shots of about 120 mg.

[00254] Dosage regimens can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus can be administered, several divided doses can be administered over time, or the dose can be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of therapeutic antibody calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. It is to be noted that proper dosing varies with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[00255] In the treatment of an A β -related disease or disorder or neuroinflammation related disease or disorder, sometimes the disease or disorder can be cured with methods provided herein, but any clinical improvement constitutes a benefit. In some embodiments, the methods provided herein reduce amyloid by an average of about 50 centiloids (CL), about 60 CL, about 70 CL, about 80 CL, about 90 CL, about 95 CL, or about 99 CL. In some embodiments, the methods provided herein reduce amyloid by an average of about 50 CL. In some embodiments, the methods provided herein reduce amyloid by an average of about 80 CL. In some embodiments, the methods provided herein reduce amyloid by an average of about 90 CL. In some embodiments, the methods provided herein reduce amyloid by an average of 50 to 100 CL, 60 to 100 CL, 70 to 100 CL, 80 to 100 CL, or 90 to 100 CL. In some embodiments, methods provided herein reduce amyloid by an average of 50 to 100 CL. In some embodiments, methods provided herein reduce amyloid by an average of 90 to 100 CL. In some embodiments, the methods provided herein reduce neuroinflammation. In some embodiments, methods provided herein reduce vasogenic edema. In some embodiments, methods provided herein prevent synaptic phagocytosis and neuronal death. In some embodiments, methods provided herein prevent the onset of AD, or delay or halt the progression AD. In some embodiments, methods provided herein ameliorate the symptoms of AD. In some embodiments, methods provided

herein ameliorate cognitive impairment. In some embodiments, methods provided herein delay the onset of cognitive impairment.

[00256] Compared to therapeutic antibodies that cross-link FcγR, the anti-CD22 antibodies disclosed herein have no or reduced vascular side effects (*i.e.*, ARIA-E, ARIA-H). Additionally, in some embodiments, methods provided herein reduce vasogenic edema.

[00257] The anti-CD22 antibodies or antigen-binding fragments disclosed herein can be administered by a variety of methods known in the art. As appreciated by those skilled in the art, the route and/or mode of administration varies depending upon the desired results. In some embodiments, the therapeutic antibody can be prepared with a carrier that protects it against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyethylene glycol (PEG), polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. *See, e.g.*, SUSTAINED AND CONTROLLED RELEASE DRUG DELIVERY SYSTEMS, J. R. Robinson ed., Marcel Dekker, Inc., New York, 1978. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and until the patient shows partial or complete amelioration of symptoms of disease.

[00258] Combination therapy using agents with different mechanisms of action can result in additive or synergetic effects. Combination therapy can allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of the agent disclosed herein. Combination therapy can decrease the likelihood that drug-resistance would develop. In some embodiments, the additional therapy results in an increase in the therapeutic index of the antibodies or antigen-binding fragments, or pharmaceutical compositions described herein. In some embodiments, the additional therapy results in a decrease in the toxicity and/or side effects of the antibodies or antigen-binding fragments or pharmaceutical compositions described herein. In some embodiments, the anti-CD22 antibodies or antigen-binding fragments, or pharmaceutical compositions described herein can be administered in combination with an additional therapy.

[00259] In some embodiments, the second therapeutic agent is an anti-Aβ antibody, an anti-CD33 antibody, a Tau aggregation inhibitor, a Tau protein modulator, a cholinesterase inhibitor, an acetylcholinesterase inhibitor, an N-methyl D-aspartate (NMDA) antagonist, a β-secretase inhibitor, or an insulin sensitizer. In some embodiments, the second therapeutic agent can be a second antibody

that suppresses the release of pro-inflammatory cytokines, or an agent that enhances microglia cell phagocytic activities.

[00260] In some embodiments, the second therapeutic agent is an anti-A β antibody. The anti-A β antibody can be selected from the group consisting of aducanumab, donanemab, gantenerumab, lecanemab, bapineuzumab, and solanezumab. In some embodiments, the second therapeutic agent is aducanumab. In some embodiments, the second therapeutic agent is donanemab. In some embodiments, the second therapeutic agent is gantenerumab.

[00261] In some embodiments, the second therapeutic agent is an anti-CD33 antibody. In some embodiments, the second therapeutic agent is selected from the group consisting of AL003 (AbbVie), gemtuzumab, lintuzumab, ozogamicin, vadastuximab talirine, and BI836858. In some embodiments, the second therapeutic agent is AL003. In some embodiments, the second therapeutic agent is gemtuzumab. In some embodiments, the second therapeutic agent is lintuzumab.

[00262] In some embodiments, the second therapeutic agent is a Tau aggregation inhibitor. In some embodiments, the second therapeutic agent is methylene blue derivative LMTX (also known as LMTM or TRx0237), or curcumin.

[00263] In some embodiments, the second therapeutic agent is a Tau protein modulator. In some embodiments, the second therapeutic agent is memantine, sodium selenate, alvocidib, seliciclib, Tideglusib, lithium, salsalate, or MK-8719.

[00264] In some embodiments, the second therapeutic agent is a cholinesterase inhibitor or an acetylcholinesterase inhibitor. In some embodiments, the second therapeutic agent is donepezil, rivastigmine, or galantamine.

[00265] In some embodiments, the second therapeutic agent is NMDA antagonist. In some embodiments, the second therapeutic agent is memantine.

[00266] The second therapeutic agent can be administered prior to, concurrently with, or subsequent to administration of the anti-CD22 antibodies or antigen-binding fragments or pharmaceutical compositions described herein. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities simultaneously. A person skilled in the art can readily determine appropriate regimens for administering a pharmaceutical composition described herein and an additional therapy in combination, including the timing and dosing of an additional agent to be used in a combination therapy, based on the needs of the subject being treated.

[00267] All papers, publications and patents cited in this specification are herein incorporated by reference as if each individual paper, publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. However, mention of any reference, article, publication, patent, patent publication, and patent application cited herein is not, and should not be taken as an acknowledgment or any form of suggestion that they constitute valid prior art or form part of the common general knowledge in any country in the world.

[00268] Unless the context indicates otherwise, it is specifically intended that the various features described herein can be used in any combination.

[00269] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

5.6 Experimental

5.6.1 Example 1: CD22 expression on microglia cells.

[00270] Using immunohistochemistry procedures, immunocytochemistry procedures, and database search, CD22 was found to be expressed on multiple types of cells, including Ramos cells, B cells, and microglia cells (*e.g.*, HMC-3 cells, and BV-2 cells).

	Ramos	Raji	Daudi	Human B cell	HMC-3	BV-2
Expression	+++	+++	+++	+++	++	+++

Ramos: Human Burkitt's lymphoma cells; HMC-3: human microglial cells; BV-2: murine microglial cells.

[00271] Consistently, as shown in the table below, the binding of various anti-CD22 antibodies to the cell lines above, including microglia cells (*e.g.*, HMC-3 cells, and BV-2 cells), were also detected.

	Ramos	Raji	Daudi	B cell	HMC-3	BV-2
SM03	+++	+++	+++	+++	+++	-
SM06	+++	+++	+++	+++	+++	-
Emab	+++	+++	+++	++	++	-
M971	++	++	++	++	+	+
Others						

+: indicate positive binding, - indicates no binding.

5.6.2 Example 2: Binding of CD22 to oligomeric A β 1-42 *in vitro*.

[00272] Octet 96e SPR system was used to analyze binding of CD22 to A β 1-42 *in vitro*. Streptavidin Biosensor was loaded with 50 μ g/mL biotinylated oligomeric A β 1-42; and the interaction with CD22

of various concentration was detected with the biosensor. The steps were as follows: first, streptavidin biosensor was loaded into 50 $\mu\text{g/mL}$ biotinylated oligomeric A β 1-42 for 4 minutes; second, biosensor was washed with kinetic buffer (PBS+ 0.02% Tween20, 0.1% BSA) for 10 seconds; third, Biosensor was loaded into various concentration of extracellular domain of CD22 (Peprotech) for 2 minutes for association. As shown in the FIG.1, CD22 bound to oligomeric A β 1-42 at a K_D of about 2.79 nM.

5.6.3 Example 3: Binding of oligomeric A β 1-42 to CD22-expressing cells.

[00273] HEK293 cells were seeded onto 6 well-plate at a density of 0.03125 million cells/ cm^2 on day 1. On day 2, HEK293 cells were transfected with Lipofectamine 3000 (Invitrogen) to overexpress full length human CD22 for 2 days. On day 4, transfected cells were incubated on ice with 1 $\mu\text{g/mL}$ FITC-A β in growth medium for 1 hour. Cells were trypsinized and analyzed by flow cytometer. As shown in FIG.2, overexpression of human CD22 in HEK293 cells increased binding of FITC-A β onto the surface of HEK293 cells, demonstrating the binding between A β and CD22 on cell surface.

5.6.4 Example 4: Binding of CD22 to oligomeric A β 1-42 on HMC-3 cells

[00274] Proximity-ligation assay (PLA) was used to evaluate the binding of CD22 to A β 1-42 on HMC-3 cells. HMC-3 cells were seeded onto 1% gelatin-coated coverslip at a density of 0.026 million cell/ cm^2 . Cells were treated with oligomeric A β on ice for 1 hour. Cells were washed once with PBS and then fixed with 4% paraformaldehyde (PFA) for 5 minutes. Cells were incubated with anti-CD22 antibody and anti-A β antibody overnight at 4°C. PLA assay was performed according to manufacturer's protocol (Duolink® Proximity Ligation Assay, Sigma-Aldrich). Fluorescent image was taken with Zeiss 710 upright microscopy. As shown in FIG.3, HMC-3 cells were encircled by dotted line. Positive signals indicated by arrows demonstrated the physical interaction between CD22 and A β .

5.6.5 Example 5: Anti-CD22 antibodies induced internalization of CD22

[00275] HMC3 cells were seeded onto 1% gelatin-coated coverslip at a density of 0.026 million cell/ cm^2 . Cells were treated with 10 $\mu\text{g/mL}$ SM03 and incubated on ice for 1 hour. Then cells were incubated at 37°C to induce internalization. At designated time points, medium was removed and cells fixed with 4% PFA. Surface CD22 expression was detected with mouse anti-human CD22 antibody (Abcam) and Alexaflour488-conjugated anti-mouse IgG antibody. As shown in FIG.4, HMC-3 cells were treated with SM03 for 3 minutes, 5 minutes, 10 minutes, 30 minutes, and 60 minutes. SM03 reduced surface CD22 expression by 20% only 3 minutes after the treatment.

5.6.6 Example 6: Internalization of anti-CD22 antibodies in human microglia cells.

[00276] HMC-3 cells were seeded onto 1% gelatin-coated coverslip at a density of 0.026 million cell/cm², and treated with FITC-SM03 for 30 mins. HMC3 cells were fixed with 4% paraformaldehyde in PBS and confocal images were acquired with Zeiss 800 microscopy. As shown in FIG.5, FITC-SM03 was internalized within 30 mins after treatment in HMC3 cells.

[00277] HMC-3 cells were seeded onto 6-well plate at a density of 0.03 million cells/cm². Cells were incubated with the indicated anti-CD22 antibodies at 10 µg/mL on ice for 2 hours. Then cells were incubated at 37°C to induce internalization. At designated time points, medium was removed and cells fixed with 4% PFA. Surface anti-CD22 antibody, namely SM03, SM06, Emab or M971, was each detected with AlexaFlour 488-conjugated anti Human IgG antibody (Invitrogen). As shown in FIG.6, different anti-CD22 antibodies showed different rates of internalization by HMC-3 cells. SM03 and SM06 induced the highest rates of internalization by HMC-3 cells.

5.6.7 Example 7: Internalization of oligomeric Aβ enhanced by anti-CD22 antibodies

[00278] HMC-3 cells were seeded onto 6-well plate at a density of 0.06 million cell/cm². HMC-3 cells were pre-treated with 1 µg/mL FITC-Aβ in growth medium for 24 hours. Cells were then treated with 10 µg/mL SM03, SM06, Emab and M971 or IgG isotype control for designated time, *e.g.*, 4 hours, 8 hours, 16 hours, and 24 hours. Cells were trypsinized and fixed with 4% PFA for 5 minutes at room temperature. Cells were stained with mouse anti-human CD22 antibody (Abcam) and FITC-Aβ internalization was analyzed with flow cytometer. As shown in FIG.7, SM03, SM06 and Emab (but not M971) showed enhanced phagocytosis of FITC-Aβ. Both SM03 and SM06 showed the highest phagocytic ability among all tested antibodies.

5.6.8 Example 8: Anti-CD22 antibodies suppressed NFκB signaling pathway in microglia cells

[00279] HMC-3 cells were seeded onto 24-well plate at a density of 0.05 million cells/cm². Cells were transfected (Invitrogen, Lipofectamine 3000) with NFκB luciferase reporter plasmid. Transfected cells were then stimulated with LPS and co-treated with different concentrations of SM03 Fab fragment. Luciferase reading was recorded 24 hours after treatment. As shown in FIG.8, SM03 Fab reduced NFκB signaling in a dose-dependent manner, demonstrating that the activation of microglia was reduced with the SM03 treatment.

5.6.9 Example 9: Anti-CD22 antibodies suppressed IL-6 secretion in microglia cells

[00280] HMC-3 cells were seeded onto 96-well plate at a density of 0.125 million cells/cm². HMC-3 cells were stimulated with LPS and co-treated with SM03 Fab fragment at different concentrations.

Twenty-four hours after treatment, medium was collected and tested with IL-6 ELISA kit (R&D systems) according to manufacturer's protocol. As shown in FIG.9, SM03 Fab reduced IL-6 secretion, further confirming that the activation of microglia was reduced with the SM03 treatment.

5.6.10 Example 10: Anti-CD22 antibodies promoted *trans* binding of 2,6 sialic acid on HMC-3 cell

[00281] HMC3 cells were seeded onto 1% gelatin-coated coverslip at a density of 0.026 million cell/cm². Cells were treated with anti-CD22 antibody for designated time. Medium was removed and cells were fixed with 4% PFA for 5 minutes at room temperature. Fixed cells were incubated with FITC-conjugated 2,6-sialic acid probe (GlycoTech) overnight at 4°C. Fluorescent images were taken with Leica DMI6000B microscopy. As shown in FIG.10, SM03 and SM06 promoted *trans* binding of 2,6-sialic acid probe in HMC-3 cells.

5.6.11 Example 11: Anti-CD22 antibodies reduce synaptic phagocytosis and neuronal death

[00282] Anti-CD22 antibodies, including SM03 and SM06, are subject to functional assays to test their activities in reducing synaptic phagocytosis and/or neuronal death. To assess synaptic phagocytosis, HMC-3 cells are co-cultured with differentiated SH-SY5Y neuronal cells. Synapse developed on differentiated SH-SY5Y is indicated by the expression of PSD-95 protein. The PSD-95 expression in the HMC-3 microglia cells can be measured using western blot or ELISA, which indicates the rate of synaptic phagocytosis by microglial cells. Treatment of SM03 and/or SM06 reduces the PSD-95 expression in HMC-3 cells, indicating that these anti-CD22 antibodies can inhibit synaptic phagocytosis by microglial cells.

[00283] To measure the neuronal death, differentiated SH-SY5Y cells are treated with the culture medium of HMC-3 cells after LPS stimulation, in the presence or absence of the designated anti-CD22 antibody. The death of SH-SY5Y is measured by MTT assay. Treatment of SM03 and/or SM06 reduces the death of SH-SY5Y.

6. Reference to Sequence Listing Submitted Electronically

[00284] This application incorporates by reference a Sequence Listing entitled "022A003WO01.XML" created on July 1, 2022 and having a size of 56,981 bytes.

What is Claimed is:

1. A method of promoting removal of beta-amyloid (A β) plaque in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.
2. A method of reducing neuroinflammation in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.
3. The method of claim 1 or 2, wherein the subject has clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma, pre-eclampsia, cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessels.
4. A method of treating an A β -related disease or disorder in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.
5. A method of treating a disease or disorder associated with neuroinflammation in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.
6. The method of claim 4 or 5, wherein the A β -related or neuroinflammation-related disease or disorder is clinical or pre-clinical Alzheimer's disease, prodromal Alzheimer's disease, Down's syndrome, clinical or pre-clinical amyloid angiopathy (CAA), Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma, pre-eclampsia, cognitive impairment, memory loss, or a vascular disorder caused by pathogenic A β peptide in blood vessels.
7. The method of claim 6, wherein the A β -related disease or disorder is Alzheimer's disease.
8. The method of any one of claims 1 to 7, wherein the antibody or antigen-binding fragment is a monoclonal antibody or antigen-binding fragment.
9. The method of any one of claims 1 to 8, wherein the antibody or antigen-binding fragment is an antibody selected from the group consisting of an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, and an IgG4 antibody.

10. The method of claim 9, wherein the antibody is an IgG1 antibody.
11. The method of any one of claims 1 to 8, wherein the antibody or antigen-binding fragment is selected from the group consisting of a Fab, a Fab', a F(ab')₂, a Fv, a scFv, a (scFv)₂, a single domain antibody (sdAb), and a heavy chain antibody (HCAb).
12. The method of any one of claims 1 to 11, wherein the antibody or antigen-binding is a chimeric antibody or antigen-binding, a humanized antibody or antigen-binding, or a human antibody or antigen-binding.
13. The method of any one of claims 1 to 12, wherein the antibody or antigen-binding fragment specifically binds to CLLNFSCYGYPIQ (SEQ ID NO:11) and VFTRSELKFSPQWSHHGKIVTC (SEQ ID NO:12) of human CD22.
14. The method of any one of claims 1 to 13, wherein the antibody or antigen-binding fragment comprises a light chain variable region (VL) comprising a VL CDR1, VL CDR2, and VL CDR3 that have the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, and SEQ ID NO:3, respectively; and a heavy chain variable region (VH) comprising a VH CDR1, VH CDR2, and VH CDR3 that have the amino acid sequence of SEQ ID NO:4, SEQ ID NO:5, and SEQ ID NO:6, respectively.
15. The method of claim [00102], wherein the antibody or antigen-binding fragment is a chimeric antibody or antigen-binding fragment.
16. The method of claim 15, wherein the VL and VH of the chimeric antibody or antigen-binding fragment have the amino acid sequences of SEQ ID NO:7 and SEQ ID NO:8, respectively.
17. The method of claim 16, wherein the chimeric antibody or antigen-binding fragment is SM03.
18. The method of claim [00102], wherein the antibody or antigen-binding fragment is a human antibody or antigen-binding fragment.
19. The method of claim 18, wherein the VL and VH of the humanized antibody or antigen-binding fragment have the amino acid sequences of SEQ ID NO:9 and SEQ ID NO:10, respectively.
20. The method of claim 19, wherein the humanized antibody or antigen-binding fragment is SM06.
21. The method of any of claims 1 to 20, wherein the antibody or antigen-binding fragment is administered intravenously, intramuscularly, subcutaneously, intracranially, intrathecally, intraventricularly, intraperitoneally, intranasally, parenterally, topically, or intradermally.
22. The method of claim 21, wherein the antibody or antigen-binding fragment is administered intravenously or subcutaneously.

23. The method of any one of claims 1 to 22, wherein the antibody or antigen-binding fragment is administered in a therapeutically effective amount within the range of 1-50 mg/kg of body weight of the subject.
24. The method of claim 23, wherein the therapeutically effective amount is about 1, about 2, about 3, about 5, about 10, about 15, or about 30 mg/kg of body weight of the subject.
25. The method of any one of claims 1 to 22, wherein the antibody or antigen-binding fragment is administered in a therapeutically effective amount at 300 - 1,200 mg per dose.
26. The method of any one of claim 1 to 25, wherein the antibody or antigen-binding fragment is administered biweekly or monthly.
27. The method of any one of claims 1 to 26, wherein the antibody or antigen-binding fragment is administered in multiple doses.
28. The method of claim 27, wherein the antibody or antigen-binding fragment is administered in multiple doses over a period of at least three months, at least six months, or at least one year.
29. The method of any one of claims 1 to 28, wherein the antibody or antigen-binding fragment is administered in combination with a second therapeutic agent.
30. The method of claim 29, wherein the second therapeutic agent is an anti-beta-amyloid antibody, an anti-CD33 antibody, a Tau aggregation inhibitor, a Tau protein modulator, a cholinesterase inhibitor, an acetylcholinesterase inhibitor, an N-methyl D-aspartate (NMDA) antagonist, a β -secretase inhibitor, or an insulin sensitizer.
31. The method of claim [00259], wherein the second therapeutic agent is an anti-beta-amyloid antibody selected from the group consisting of aducanumab, donanemab, gantenerumab, lecanemab, bapineuzumab, and solanezumab.
32. The method of claim 28, wherein the second therapeutic agent is an anti-CD33 antibody selected from the group consisting of AL003, gemtuzumab, lintuzumab, ozogamicin, vadastuximab talirine, and BI836858.
33. The method of any one of claims 1 to [00261], wherein the subject is a human subject.
34. A method of inducing internalization of A β by a microglia cell, comprising contacting the microglia cell with an effective amount of an antibody or antigen-binding fragment thereof that specifically binds to CD22, wherein the antibody or antigen-binding fragment (a) promotes cis-trans conversion of CD22 and/or (b) induces internalization of CD22.

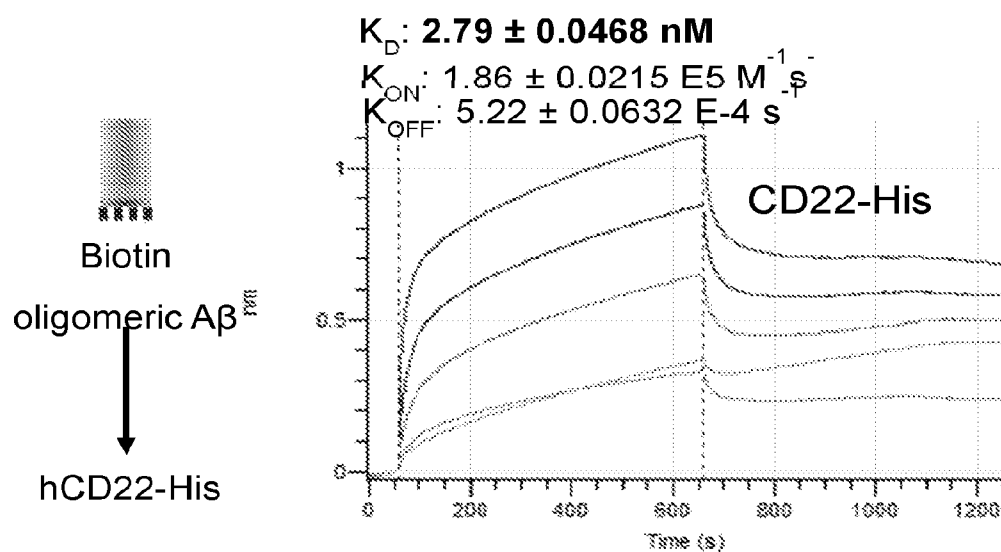


FIG.1

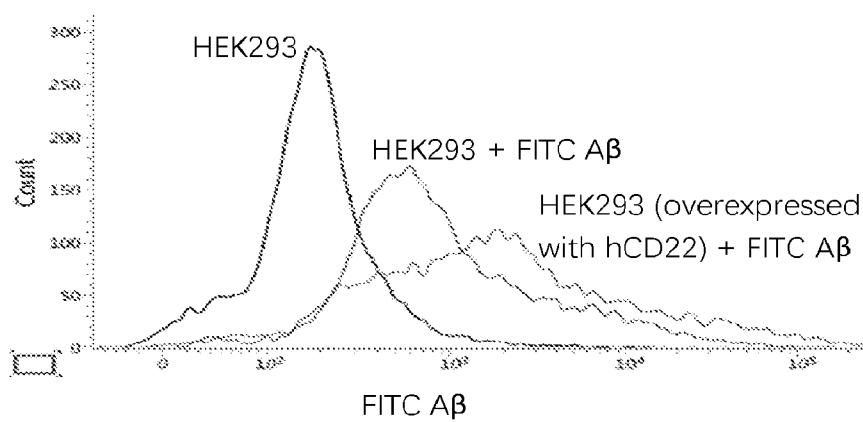


FIG.2

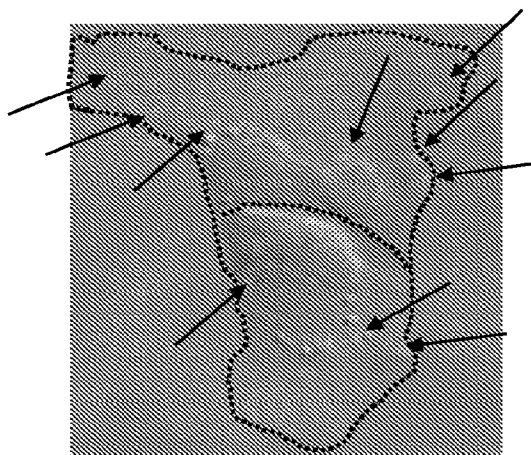


FIG.3

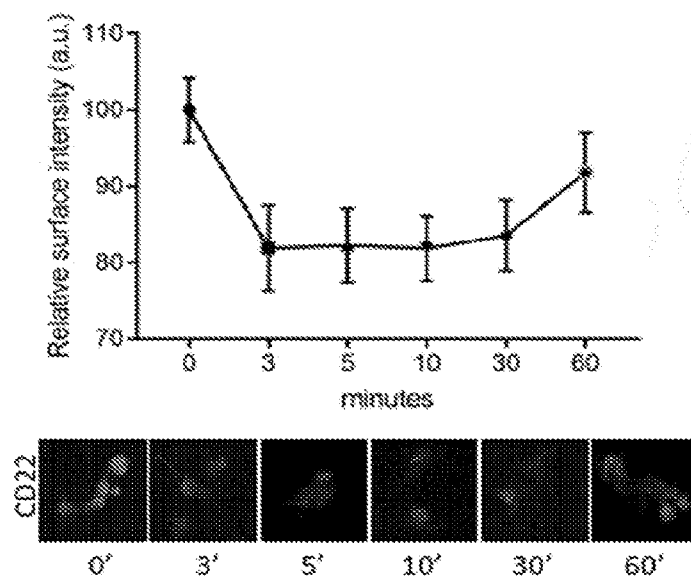


FIG.4

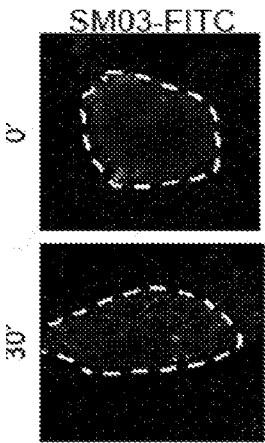


FIG.5

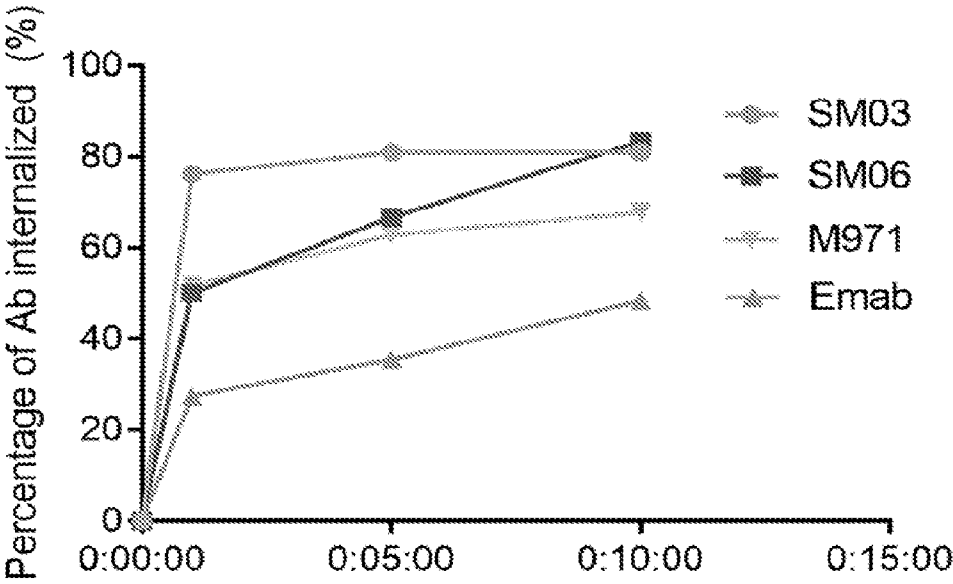


FIG.6

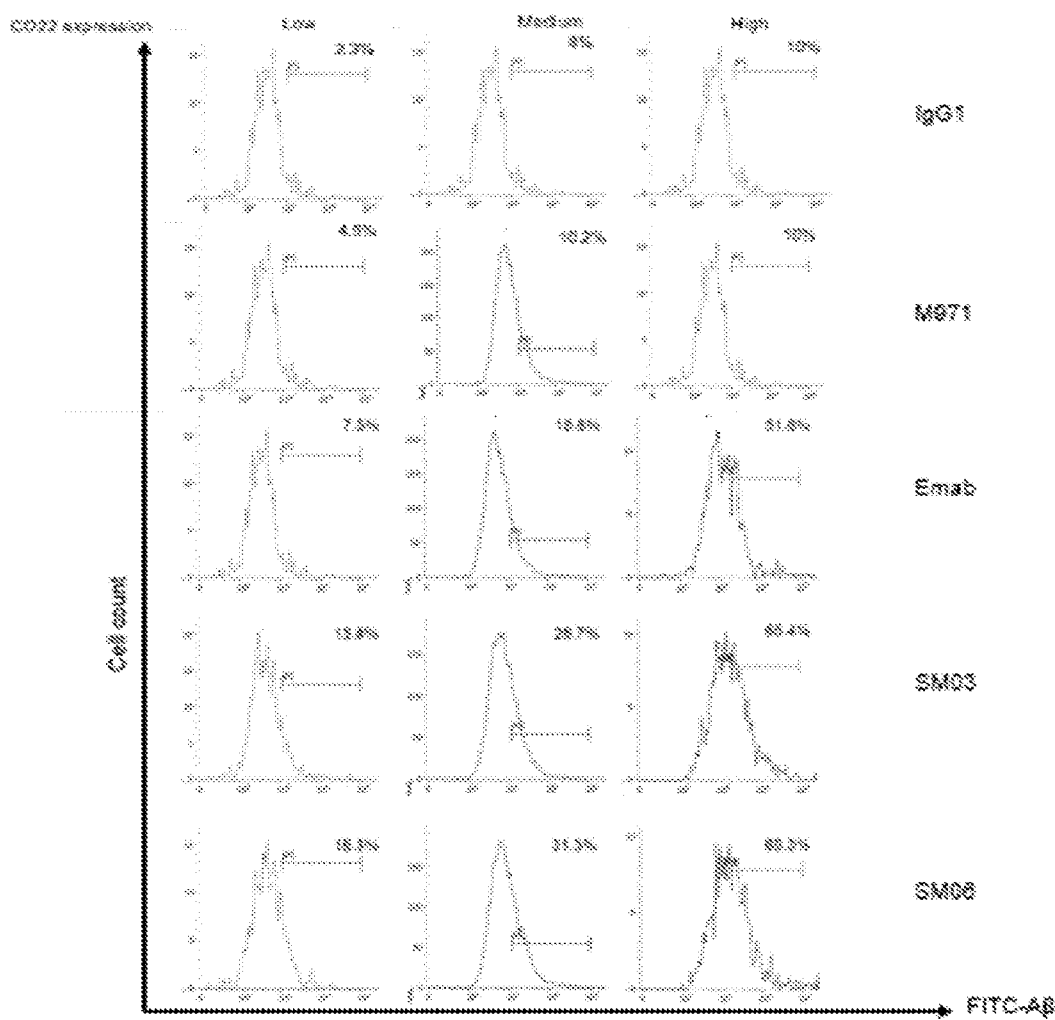


FIG.7

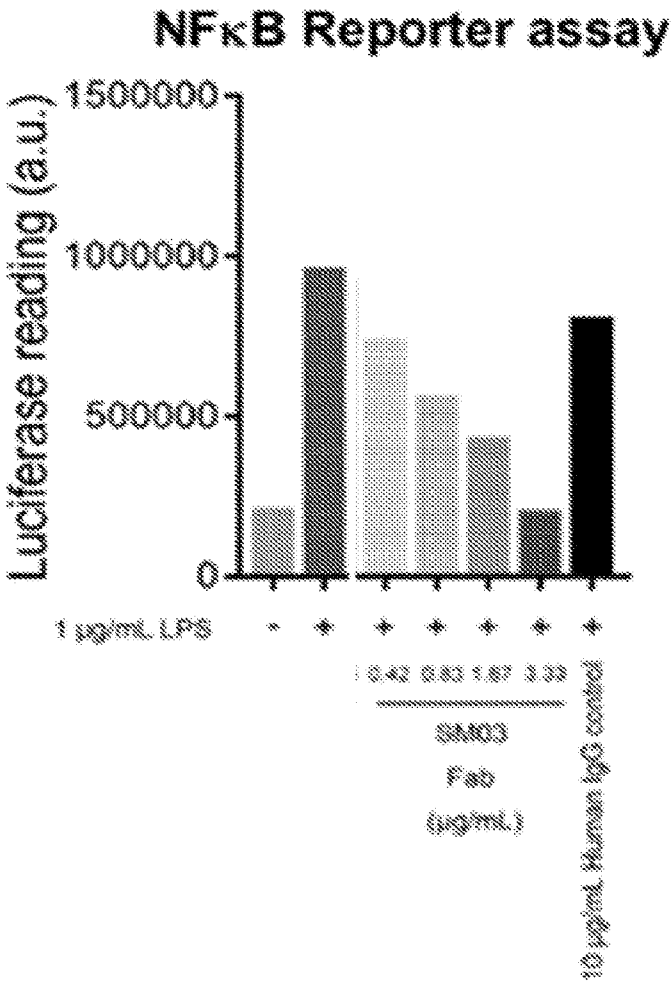


FIG.8

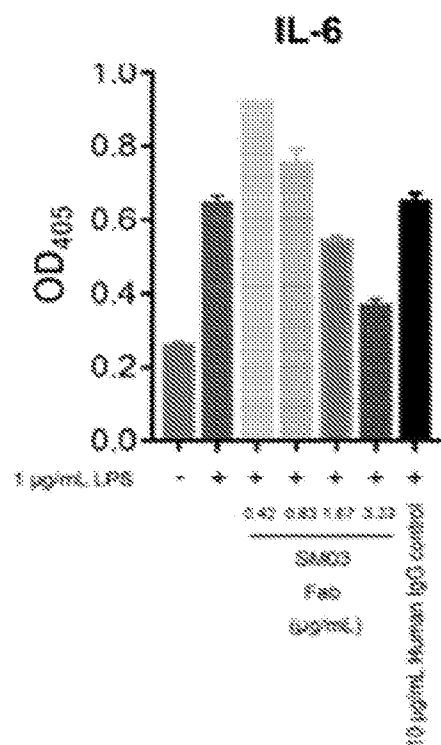


FIG.9

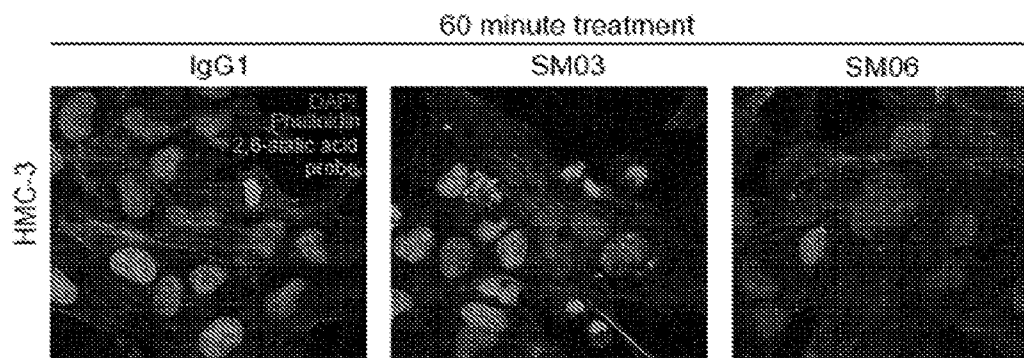


FIG.10