(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 December 2021 (16.12.2021)

WIPO | PCT

(10) International Publication Number
WO 2021/252157 A1

(51) International Patent Classification:
A61P 25/28 (2006.01) *A61P 37/02* (2006.01)
A61P 25/02 (2006.01)

(21) International Application Number:
PCT/US2021/033226

(22) International Filing Date:
19 May 2021 (19.05.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/037,414 10 June 2020 (10.06.2020) US

(71) Applicant: **CRYSTAL BIOSCIENCE INC.** [US/US]; 3911 Sorrento Valley Boulevard, Suite 110, San Diego, California 92121 (US).

(72) Inventors: **CHING, Kathryn**; c/o Crystal Bioscience Inc., 3911 Sorrento Valley Boulevard, Suite 110, San Diego, California 92121 (US). **BERG, Kimberley**; c/o Crystal Bioscience Inc., 3911 Sorrento Valley Boulevard, Suite 110, San Diego, California 92121 (US).

(74) Agent: **KEDDIE, James S.**; Bozicevic, Field & Francis LLP, 201 Redwood Shores Parkway, Suite 200, Redwood City, California 94065 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: ANTI-BDNF ANTIBODIES AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure provides antibodies that specifically bind to and in some cases inhibit human brain-derived neurotrophic factor (BDNF). Pharmaceutical compositions including the same are also provided, as well as a method for inhibiting BDNF signaling. The antibodies find use in a variety of treatment, diagnostic, and monitoring applications, which are also described.

WO 2021/252157 A1

ANTI-BDNF ANTIBODIES AND METHODS OF USE THEREOF

CROSS-REFERENCING

This application claims the benefit of U.S. provisional application serial no. 63/037,414, filed on June 10, 2020, which application is incorporated by reference in its entirety.

BACKGROUND

Brain-derived neurotrophic factor (BDNF), is a small soluble protein with molecular weight of 13kDa for the monomer (27kDa as homodimer) that belongs to the neurotrophin family of growth factors. It shares amino acid sequence homology to other family members including Nerve Growth Factor (NGF), Neurotrophin-3 (NT-3) and Neurotrophin-4 (NT-4) and is composed of a highly homologous structure containing antiparallel β strands and cysteine residues in a cystine knot motif. BDNF is important in developmental neurobiology where it controls aspects of survival, differentiation and proliferation of neurons in both the peripheral and central nervous systems. Furthermore, in adulthood, BDNF controls aspects of neuronal function, where it regulates synapse formation and synaptic plasticity. Although widely expressed in a number of tissues, BDNF is highly abundant in the brain and its activity is linked to processes such as long term potentiation that underlies learning and memory. BDNF mutant (BDNF -/-) mice suffer from developmental defects and usually fail to survive beyond the second postnatal week. Mice lacking BDNF display sensory neuron losses particularly in the vestibular and nodose-petrosal ganglion, that affect coordination and balance, suggesting that BDNF plays an important role in normal neural development. The physiological actions of BDNF are mediated via interaction with two types of receptors; the high affinity tyrosine receptor kinase B (TrkB) and p75NTR also known as low-affinity nerve growth factor receptor (LNGFR).

BDNF engagement of the TrkB receptor results in the dimerization of the TrkB receptor, leading to autophosphorylation of tyrosine residues in the cytoplasmic domain and enhanced tyrosine kinase activity of the receptor. This yields docking sites for adapter proteins containing phosphotyrosine-binding (PTB) or src- homology-2 (SH-2) motif that couple the receptor to multiple intracellular signaling cascades such as Ras/ERK (extracellular signal-regulated kinase), PI3K (phosphatidylinositol-3-kinase) and PLC-γ (phospholipase C γ). These pathways are involved in different aspects of neurone development and cell function including cell survival, differentiation, neurite outgrowth and synapse formation. The lower affinity p75NTR on the other hand, is a member of the tumour necrosis receptor superfamily. Unlike TrkB, it lacks intrinsic catalytic activity and contains a death domain in the cytoplasmic sequence. All members of the neurotrophin family activate p75NTR with similar affinities

and ligand engagement leads to activation of several intracellular signal transduction pathways, including nuclear factor-κB (NF-κB), Jun kinase and sphingo-myelin hydrolysis. Trk-p75NTR interaction has been proposed to critically regulate Trk receptor signalling and furthermore enhance the ligand specificity of Trk receptors. The functional role of p75NTR is diverse and is implicated in both pro- and antitrophic processes, including neurite outgrowth and ligand mediated apoptosis.

Dysregulation in BDNF levels has been documented in a number of human disease conditions including a variety of neurological disorders, joint disease, peripheral nerve damage, intervertebral disc degeneration and visceral conditions such as inflammatory bowel syndrome, chronic pancreatitis and overactive bladder. Moreover, correlations between peripheral BDNF levels and pain or disease severity have been documented. Accordingly, there is a need to provide agents that specifically and preferably selectively recognize and interact with BDNF and dampen or inhibit BDNF signaling through its receptor and to provide for therapeutic use of such agents particularly in conditions associated with BDNF.

SUMMARY OF THE INVENTION

The present disclosure provides antibodies that specifically bind to and in some cases inhibit human brain-derived neurotrophic factor (BDNF). The antibodies find use in a variety of treatment, diagnostic, and monitoring applications, which are also described.

In some embodiments, the antibody may comprise: (a) a variable domain comprising: i. heavy chain CDR1, CDR2 and CDR3 regions that are identical to the heavy chain CDR1, CDR2 and CDR3 regions of an antibody selected from Fig. 1; and ii. light chain CDR1, CDR2 and CDR3 regions that are identical to the light chain CDR1, CDR2 and CDR3 regions of an antibody selected from Fig. 2; or (b) a variant of said variable domain of (a) that is otherwise identical to said antibody variable domain except for up to 10 (e.g., up to 9, 8, 7, 6, 5, 4, 3, 2, or 1) amino acid substitutions in the collective CDR regions of the variable domain of (a).

In some embodiments, the antibody comprises: a heavy chain variable domain comprising an amino acid sequence that is at least 90% (e.g., at least 95%) identical to the amino acid sequence of the heavy chain variable domain of an antibody selected from Fig. 1; and a light chain variable domain comprising an amino acid sequence that is at least 90% (e.g., at least 95%) identical to the light chain variable domain of the antibody, selected from Fig. 2.

Human and murine BDNF are very similar at the protein level and, as such, it has been challenging to generate inhibitory antibodies using mice and other mammals. In the present study, chicken was used as an alternative host in order to generate a more diverse panel of antibodies. There is greater evolutionary distance between humans and chickens compared to humans and other mammals such as mice. This evolutionary distance allows chickens to produce a more vigorous and diverse

immune response when challenged with human proteins. Another major advantage of chicken immunization is the generation of antibodies that recognize “pan-mammalian” epitopes; such antibodies are difficult or impossible to generate in mammalian hosts. An additional advantage of chicken antibodies comes with the broad species cross-reactivity, obviating the need to generate surrogate antibodies for the purpose of experimentation in various disease models.

The present disclosure also provides a method of treating BDNF-related disorder. In one embodiment, a therapeutically effective amount of subject antibody is administered to a subject in need thereof. Methods of administration and delivery are also provided.

Thus, the present antibodies have a number of advantages over antibodies that are produced in mammalian hosts.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1: Heavy chain sequences. Fig. 1 provides an alignment of the amino acid sequence of several anti-BDNF antibody heavy chain variable regions, in which the complementarity determining regions (CDRs) as defined by the IMGT system are indicated by boxes. This alignment used the MUSCLE alignment tool (Edgar, Nucl. Acids Res 2004 32: 1792–97). From top to bottom: SEQ ID NOS: 1, 1, 2-15, 15, 16-18, 18, 19, 20, 21, 21, 22-25, 25, 26-37, 31, 38, 30, 39, 40, 41, 41, 43, 42, 43, 44-48, 47, 47, 49, 49, 49, 49, 50, 51, 50, 52, 53, 51, 55, 55, 51, 50, 54, 55, 56-70.

Fig. 2: Light chain sequences. Fig. 1 provides an alignment of the amino acid sequence of several anti- BDNF antibody light chain variable regions, in which the complementarity determining regions (CDRs) as defined by the IMGT system are indicated by boxes. This alignment used the MUSCLE alignment tool (Edgar, Nucl. Acids Res 2004 32: 1792–97). From top to bottom: SEQ ID NOS: 71-99, 99, 100-159.

FIG. 3: Phylogenetic tree of chicken derived anti- BDNF antibodies.

DEFINITIONS

The terms "antibodies" and “immunoglobulin” include antibodies or immunoglobulins of any isotype, fragments of antibodies which retain specific binding to antigen, including, but not limited to, Fab, Fv, scFv, and Fd fragments, chimeric antibodies, humanized antibodies, single-chain antibodies, and fusion proteins comprising an antigen-binding portion of an antibody and a non-antibody protein. The antibodies may be detectably labeled, *e.g.*, with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, and the like. The antibodies may be further conjugated to other moieties, such as members of specific binding pairs, *e.g.*, biotin (member of biotin-avidin specific

binding pair), and the like. The antibodies may also be bound to a solid support, including, but not limited to, polystyrene plates or beads, and the like. Also encompassed by the term are Fab', Fv, $F(ab')_2$, and or other antibody fragments that retain specific binding to antigen, and monoclonal antibodies. An antibody may be monovalent or bivalent.

"Antibody fragments" comprise a portion of an intact antibody, for example, the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 (1995)); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRS of each variable domain interact to define an antigen-binding site on the surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The “Fab” fragment also contains the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH_1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2.

"Single-chain Fv" or "sFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the

sFv to form the desired structure for antigen binding. For a review of sFv, see *Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994)*.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and *Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993).*

As used herein, the term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as a dissociation constant (Kd). Affinity can be at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1000-fold greater, or more, than the affinity of an antibody for unrelated amino acid sequences. Affinity of an antibody to a target protein can be, for example, from about 100 nanomolar (nM) to about 0.1 nM, from about 100 nM to about 1 picomolar (pM), or from about 100 nM to about 1 femtomolar (fM) or more. As used herein, the term “avidity” refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms “immunoreactive” and “preferentially binds” are used interchangeably herein with respect to antibodies and/or antigen-binding fragments.

The term “binding” refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. An anti-BDNF antibody binds specifically to an epitope within a BDNF polypeptide. Non-specific binding would refer to binding with an affinity of less than about 10^{-7} M, e.g., binding with an affinity of 10^{-6} M, 10^{-5} M, 10^{-4} M, etc.

As used herein, the term “CDR” or “complementarity determining region” is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. CDRs have been described by Kabat et al., J. Biol. Chem. 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, “Sequences of proteins of immunological interest” (1991); by Chothia et al., J. Mol. Biol. 196:901-917 (1987); and MacCallum et al., J. Mol. Biol. 262:732-745 (1996), and Lefranc, M.-P. et al., Dev. Comp. Immunol., 2003 27: 55-77, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The CDRs

indicated in Figs. 1 and 2 are defined by the IMGT method described in Lefranc et al. (Dev. Comp. Immunol., 2003 27: 55-77). However, as indicated below, the Chothia, Kabat and MacCallum method could also be used to define the CDRs.

Table 1: CDR Definitions

	Kabat[1]	Chothia[2]	MacCallum[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	53-55	47-58
V_H CDR3	95-102	96-101	93-101
V_L CDR1	24-34	26-32	30-36
V_L CDR2	50-56	50-52	46-55
V_L CDR3	89-97	91-96	89-96

[1] Residue numbering follows the nomenclature of Kabat et al., *supra*
[2] Residue numbering follows the nomenclature of Chothia et al., *supra*
[3] Residue numbering follows the nomenclature of MacCallum et al., *supra*

As used herein, the term “framework” when used in reference to an antibody variable region is intended to mean all amino acid residues outside the CDR regions within the variable region of an antibody. A variable region framework is generally a discontinuous amino acid sequence between about 100-120 amino acids in length but is intended to reference only those amino acids outside of the CDRs. As used herein, the term “framework region” is intended to mean each domain of the framework that is separated by the CDRs.

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, the antibody will be purified (1) to greater than 90%, greater than 95%, or greater than 98%, by weight of antibody as determined by the Lowry method, for example, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or nonreducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. In some instances, isolated antibody will be prepared by at least one purification step.

As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein,

covers any treatment of a disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

The terms "individual," "subject," "host," and "patient," used interchangeably herein, refer to a mammal, including, but not limited to, murines (rats, mice), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc.

A "therapeutically effective amount" or "efficacious amount" refers to the amount of an anti-BDNF antibody that, when administered to a mammal or other subject for treating a disease, is sufficient to effect such treatment for the disease. The "therapeutically effective amount" will vary depending on the anti- BDNF antibody, the disease and its severity and the age, weight, etc., of the subject to be treated.

A "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as polynucleotides. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

As used herein, the term "collectively" in the context of a variant of an antibody variable domain that is otherwise identical to the antibody variable domain except for a defined number of amino acid substitutions "in the collective CDR regions of the antibody variable domain", indicates that the number of amino acid substitutions is counted using all six CDRs. Explained by example, if the variant has 5 amino acid substitutions relative to the antibody variable domain, then the six CDRs of the variant, combined, have a total of 5 amino acid substitutions relative to the antibody variable domain. This phrase is not intended to mean that each CDR has a defined number of amino acid substitutions.

Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within

the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, 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 invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes a plurality of such antibodies and reference to "the anti-BDNF antibody" includes reference to one or more anti- BDNF antibodies and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements or use of a "negative" limitation.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

The present disclosure provides antibodies that bind to human BDNF. The antibodies are useful in various treatment, diagnostic, and monitoring applications, which are also provided.

In some embodiments, a subject antibody specifically binds BDNF from humans and other mammals, e.g., monkey and mouse.

For example, a subject antibody may bind to human, monkey and/or mouse BDNF with an affinity of at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, at least about 10^{-10} M, at least about 10^{-11} M, or at least about 10^{-12} M, or greater than 10^{-12} M. A subject antibody binds to an epitope present on human, monkey and/or mouse BDNF with an affinity of from about 10^{-7} M to about 10^{-8} M, from about 10^{-8} M to about 10^{-9} M, from about 10^{-9} M to about 10^{-10} M, from about 10^{-10} M to about 10^{-11} M, or from about 10^{-11} M to about 10^{-12} M, or greater than 10^{-12} M.

A subject antibody can in some embodiments reduce binding of BDNF to a BDNF receptor, such as the LNGFR (for low-affinity nerve growth factor receptor, also known as p75), tropomyosin receptor kinase B (TrkB), or a neurotransmitter receptor, e.g., alpha-7 nicotinic receptor. For example, in some embodiments a subject antibody can reduce binding of BDNF to a BDNF receptor by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the degree of binding between BDNF and the BDNF receptor in the absence of the antibody.

In some embodiments a subject antibody may reduce BDNF activity, i.e., signaling in response to BDNF. For example, in some embodiments a subject antibody may reduce BDNF signaling by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to BDNF signaling in the absence of the antibody.

The term "antibody" refers to a protein comprising one or more (e.g., one or two) heavy chain variable regions (VH) and/or one or more (e.g., one or two) light chain variable regions (VL), or subfragments thereof capable of binding an epitope. The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions (CDR)", interspersed with regions that are more conserved, termed "framework regions (FR)". The extent of the FR and CDRs has been precisely defined (see, Kabat, et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242; Chothia et al. (1987) J. Mol. Biol. 196: 901-917). A VH can comprise three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Similarly, a VL can comprise three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The VH or VL chain of an antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy and two light chains, wherein the heavy and light chains are interconnected by, for example, disulfide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable regions of the heavy and light chains comprise binding regions that interact with antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues and factors, including various cells of the immune system and the first component of the complement system. The term "antibody" includes intact immunoglobulins of types IgA, IgG, IgE, IgD, IgM and subtypes thereof. In some embodiments, a subject antibody is an IgG isotype.

As used herein the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes; and numerous immunoglobulin variable region genes. Full-length immunoglobulin light chains (about 25 kD or 214 amino acids) are encoded by a variable region gene at the N-terminus (about 110 amino acids) and a kappa or lambda constant region at the C-terminus. Full-length immunoglobulin heavy chains (about 50 kD or 446 amino acids) are encoded by a variable region gene at the N-terminus (about 116 amino acids) and one of the other aforementioned constant region genes at the C-terminus, e.g. gamma (encoding about 330 amino acids). In some embodiments, a subject antibody comprises full-length immunoglobulin heavy chain and a full-length immunoglobulin light chain.

In some embodiments, a subject antibody does not comprise a full-length immunoglobulin heavy chain and a full-length immunoglobulin light chain, and instead comprises antigen-binding fragments of a full-length immunoglobulin heavy chain and/or a full-length immunoglobulin light chain. In some embodiments, the antigen-binding fragments are contained on separate polypeptide chains; in other embodiments, the antigen-binding fragments are contained within a single polypeptide chain. The term "antigen-binding fragment" refers to one or more fragments of a full-length antibody that are capable of specifically binding to BDNF as described above. Examples of binding fragments include (i) a Fab fragment (a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment (a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) an Fd fragment (consisting of the VH and CH1 domains); (iv) an Fv fragment (consisting of the VH and VL domains of a single arm of an antibody); (v) a dAb fragment (consisting of the VH domain); (vi) an isolated CDR; (vii) a single chain Fv (scFv) (consisting of the VH and VL domains of a single arm of an antibody joined by a synthetic linker using recombinant means such that the VH and VL domains pair to form a monovalent molecule); (viii) diabodies (consisting of two scFvs in which the VH and VL domains are joined such that they do not pair to form a monovalent molecule; the VH of each one of the scFv pairs with the VL domain of the other scFv to form a bivalent molecule); (ix) bi-specific antibodies (consisting of at least two antigen binding regions, each region binding a different epitope). In some embodiments, a subject antibody fragment is a Fab fragment. In some embodiments, a subject antibody fragment is a single-chain antibody (scFv).

In some embodiments, a subject antibody is a recombinant or modified antibody, e.g., a chimeric, humanized, deimmunized or an *in vitro* generated antibody. The term "recombinant" or "modified" antibody as used herein is intended to include all antibodies that are prepared, expressed, created, or isolated by recombinant means, such as (i) antibodies expressed using a recombinant expression vector transfected into a host cell; (ii) antibodies isolated from a recombinant, combinatorial

antibody library; (iii) antibodies isolated from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes; or (iv) antibodies prepared, expressed, created, or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant antibodies include humanized, CDR grafted, chimeric, deimmunized, and *in vitro* generated antibodies; and can optionally include constant regions derived from human germline immunoglobulin sequences.

In some embodiments, a subject antibody comprises: a variable domain comprising: a) a heavy chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the sequence of a heavy chain CDR1 region of an antibody selected from the antibodies shown in Figs. 1 and 2; ii. a CDR2 region that is identical in amino acid sequence to the sequence of a heavy chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the sequence of a heavy chain CDR3 region of the selected antibody; and b) a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the sequence of a light chain CDR1 region of the selected antibody; ii. a CDR2 region that is identical in amino acid sequence to that of a light chain CDR2 region of the selected antibody sequence; and iii. a CDR3 region that is identical in amino acid sequence to that of a light chain CDR3 region of the selected antibody; wherein the antibody specifically binds human, monkey, rat and/or mouse BDNF.

In certain embodiments, an antibody comprising: (a) a variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the heavy chain CDR1 region of an antibody selected from the antibodies shown in Figs. 1 and 2; ii. a CDR2 region that is identical in amino acid sequence to the heavy chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the heavy chain CDR3 region of the selected antibody; and a light chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to the light chain CDR1 region of the selected antibody; ii. a CDR2 region that is identical in amino acid sequence to the light chain CDR2 region of the selected antibody; and iii. a CDR3 region that is identical in amino acid sequence to the light chain CDR3 region of the selected antibody; or (b) a variant of the variable domain of part (a) that is otherwise identical to the variable domain of part (a) except for up to 10 (e.g., up to 9, 8, 7, 6, 5, 4, 3, 2, or 1) amino acid substitutions in the collective CDR regions of the variable domain of (a), wherein the antibody binds to BDNF.

In some embodiments, the antibody may contain only a heavy chain variable domain described herein. In these embodiments, the antibody may be a “heavy chain only” antibody.

The heavy and light chain sequences disclosed herein can be analyzed via lineage analysis in order obtain consensus sequences for the CDRs. Groups of sequences that are believed to be related by lineage can be grouped together using the phylogenetic tree of Fig. 3. These sequences are already clustered together in Figs. 1 and 2. Analysis of these sequences reveals which amino acid positions can

tolerate amino acid substitutions and, as such, can be used to make variants of the present antibodies that have, for example, amino acid substitutions in the CDRs.

In some embodiments, a subject antibody (e.g., a subject antibody that specifically binds BDNF) may comprises: a) a light chain region comprising: i) one, two, or three complementarity determining regions (CDRs) from a light chain variable region sequence of a selected anti-BDNF antibody; and ii) a light chain framework region, e.g., a framework region from a human immunoglobulin light chain; and b) a heavy chain region comprising: i) one, two, or three CDRs from the heavy chain variable region sequence of a selected antibody; and ii) a heavy chain framework region, e.g., a framework region from a human immunoglobulin heavy chain.

A subject antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a sequence depicted in Fig. 1 and set forth in SEQ ID NOS: 1, 1, 2-15, 15, 16-18, 18, 19, 20, 21, 21, 22-25, 25, 26-37, 31, 38, 30, 39, 40, 41, 41, 43, 42, 43, 44-48, 47, 47, 49, 49, 49, 49, 50, 51, 50, 52, 53, 51, 55, 55, 51, 50, 54, 55, 56-70. A subject antibody can comprise a heavy chain variable region comprising one, two, or three of the heavy chain complementarity determining regions (CDRs) of a selected anti-BDNF antibody.

A subject antibody can comprise a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence depicted in Fig. 2 and set forth in SEQ ID NOS: 71-99, 99, 100-159. A subject antibody can comprise a light chain variable region comprising one, two, or three of the light chain CDRs of a selected anti-BDNF antibody.

In some embodiments, a subject antibody comprises anti-BDNF antibody heavy chain CDRs and anti-BDNF antibody light chain CDRs in a single polypeptide chain, e.g., in some embodiments, a subject antibody is a scFv. In some embodiments, a subject antibody comprises, in order from N-terminus to C-terminus: a first amino acid sequence of from about 5 amino acids to about 25 amino acids in length; an light chain CDR1 of a selected anti-BDNF antibody; a second amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a light chain CDR2 of a selected anti-BDNF antibody; a third amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a light chain CDR3 of a selected anti-BDNF antibody; a fourth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a heavy chain CDR1 of a selected anti-BDNF antibody; a fifth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a heavy chain CDR2 of a selected anti-BDNF antibody; a sixth amino acid sequence of from about 5 amino acids to about 25 amino acids in length; a heavy chain CDR3 of a selected anti-BDNF antibody; and a seventh amino acid sequence of from about 5 amino acids to about 25 amino acids in length.

In some embodiments, a subject antibody may comprise, in order from N-terminus to C-terminus: a light chain FR1 region; a light chain CDR1 of a selected anti-BDNF antibody; a light chain FR2 region; a light chain CDR2 of a selected anti-BDNF antibody; a light chain FR3 region; a light chain CDR3 of a selected anti-BDNF antibody; optionally a light chain FR4 region; a linker region; optionally a heavy chain FR1 region; a heavy chain CDR1 of a selected anti-BDNF antibody; a heavy chain FR2 region; a heavy chain CDR2 of a selected anti-BDNF antibody; a heavy chain FR3 region; a heavy chain CDR3 of a selected anti-BDNF antibody; and a heavy chain FR4 region. In some of these embodiments, each of the FR regions is a human FR region. The linker region can be from about 5 amino acids to about 50 amino acids in length, e.g., from about 5 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, or from about 45 aa to about 50 aa in length.

Linkers suitable for use a subject antibody include "flexible linkers". If present, the linker molecules are generally of sufficient length to permit some flexible movement between linked regions. The linker molecules are generally about 6-50 atoms long. The linker molecules may also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules which can bind to polypeptides may be used in light of this disclosure.

Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, or 7 amino acids.

Exemplary flexible linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $GSGGS_n$ (SEQ ID NO: 160) and $GGGS_n$ (SEQ ID NO: 161), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are of interest since both of these amino acids are relatively unstructured, and therefore may serve as a neutral tether between components. Glycine polymers are of particular interest since glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary flexible linkers include, but are not limited GGSG (SEQ ID NO: 162), GGSGG (SEQ ID NO: 163), GSGSG (SEQ ID NO: 164), GSGGG (SEQ ID NO: 165), GGGSG (SEQ ID NO: 166), GSSSG (SEQ ID NO: 167), and the like. The ordinarily skilled artisan will recognize that design of a peptide conjugated to any elements described above can include linkers that are all or partially flexible,

such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure.

In some embodiments, a subject antibody may be “humanized.” The term "humanized antibody" refers to an antibody comprising at least one chain comprising variable region framework residues substantially from a human antibody chain (referred to as the acceptor immunoglobulin or antibody) and at least one CDR substantially from a non-human antibody (referred to as the donor immunoglobulin or antibody). See, Queen et al., Proc. Natl. Acad. Sci. USA 86:10029 10033 (1989), U.S. Pat. No. 5,530,101, U.S. Pat. No. 5,585,089, U.S. Pat. No. 5,693,761, WO 90/07861, and U.S. Pat. No. 5,225,539. The constant region(s), if present, can also be substantially or entirely from a human immunoglobulin. In some embodiments, a subject antibody comprises one or more of the CDRs and one or more FR regions from a human antibody. Methods of making humanized antibodies are known in the art. See, e.g., U.S. Patent No. 7,256,273.

The substitution of mouse CDRs into a human variable domain framework can result in retention of their correct spatial orientation where, e.g., the human variable domain framework adopts the same or similar conformation to the mouse variable framework from which the CDRs originated. This can be achieved by obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies. See Kettleborough et al., Protein Engineering 4:773 (1991); Kolbinger et al., Protein Engineering 6:971 (1993).

Having identified the complementarity determining regions of the murine donor immunoglobulin and appropriate human acceptor immunoglobulins, the next step is to determine which, if any, residues from these components should be substituted to optimize the properties of the resulting humanized antibody. In general, substitution of human amino acid residues with murine should be minimized, because introduction of murine residues increases the risk of the antibody eliciting a human-anti-mouse-antibody (HAMA) response in humans. Art-recognized methods of determining immune response can be performed to monitor a HAMA response in a particular patient or during clinical trials. Patients administered humanized antibodies can be given an immunogenicity assessment at the beginning and throughout the administration of said therapy. The HAMA response is measured, for example, by detecting antibodies to the humanized therapeutic reagent, in serum samples from the patient using a method known to one in the art, including surface plasmon resonance technology (BIACORE) and/or solid-phase ELISA analysis. In many embodiments, a subject humanized antibody does not substantially elicit a HAMA response in a human subject.

Certain amino acids from the human variable region framework residues are selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. The unnatural juxtaposition of murine CDR regions with human variable framework region can result in unnatural conformational restraints, which, unless corrected by substitution of certain amino acid residues, lead to loss of binding affinity.

The selection of amino acid residues for substitution can be determined, in part, by computer modeling. Computer hardware and software for producing three-dimensional images of immunoglobulin molecules are known in the art. In general, molecular models are produced starting from solved structures for immunoglobulin chains or domains thereof. The chains to be modeled are compared for amino acid sequence similarity with chains or domains of solved three-dimensional structures, and the chains or domains showing the greatest sequence similarity is/are selected as starting points for construction of the molecular model. Chains or domains sharing at least 50% sequence identity are selected for modeling, and preferably those sharing at least 60%, 70%, 80%, 90% sequence identity or more are selected for modeling. The solved starting structures are modified to allow for differences between the actual amino acids in the immunoglobulin chains or domains being modeled, and those in the starting structure. The modified structures are then assembled into a composite immunoglobulin. Finally, the model is refined by energy minimization and by verifying that all atoms are within appropriate distances from one another and that bond lengths and angles are within chemically acceptable limits.

CDR and framework regions are as defined by Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, Md., 1987 and 1991). An alternative structural definition has been proposed by Chothia et al., J. Mol. Biol. 196:901 (1987); Nature 342:878 (1989); and J. Mol. Biol. 186:651 (1989) (collectively referred to as "Chothia"). When framework residues, as defined by Kabat, supra, constitute structural loop residues as defined by Chothia, supra, the amino acids present in the mouse antibody may be selected for substitution into the humanized antibody. Residues which are "adjacent to a CDR region" include amino acid residues in positions immediately adjacent to one or more of the CDRs in the primary sequence of the humanized immunoglobulin chain, for example, in positions immediately adjacent to a CDR as defined by Kabat, or a CDR as defined by Chothia (See e.g., Chothia and Lesk JMB 196:901 (1987)). These amino acids are particularly likely to interact with the amino acids in the CDRs and, if chosen from the acceptor, to distort the donor CDRs and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit et al., Science, 233:747 (1986)) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

In some embodiments, a subject antibody comprises scFv multimers. For example, in some embodiments, a subject antibody is an scFv dimer (e.g., comprises two tandem scFv ($scFv_2$)), an scFv

trimer (e.g., comprises three tandem scFv ($scFv_3$)), an scFv tetramer (e.g., comprises four tandem scFv ($scFv_4$)), or is a multimer of more than four scFv (e.g., in tandem). The scFv monomers can be linked in tandem via linkers of from about 2 amino acids to about 10 amino acids in length, e.g., 2 aa, 3 aa, 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, or 10 aa in length. Suitable linkers include, e.g., $(Gly)_x$, where x is an integer from 2 to 10. Other suitable linkers are those discussed above. In some embodiments, each of the scFv monomers in a subject scFV multimer is humanized, as described above.

In some embodiments, a subject antibody comprises a constant region of an immunoglobulin (e.g., an Fc region). The Fc region, if present, can be a human Fc region. If constant regions are present, the antibody can contain both light chain and heavy chain constant regions. Suitable heavy chain constant region include CH1, hinge, CH2, CH3, and CH4 regions. The antibodies described herein include antibodies having all types of constant regions, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. An example of a suitable heavy chain Fc region is a human isotype IgG1 Fc. Light chain constant regions can be lambda or kappa. A subject antibody (e.g., a subject humanized antibody) can comprise sequences from more than one class or isotype. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab' F(ab')2, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

In some embodiments, a subject antibody comprises a free thiol (-SH) group at the carboxyl terminus, where the free thiol group can be used to attach the antibody to a second polypeptide (e.g., another antibody, including a subject antibody), a scaffold, a carrier, etc.

In some embodiments, a subject antibody comprises one or more non-naturally occurring amino acids. In some embodiments, the non-naturally encoded amino acid comprises a carbonyl group, an acetyl group, an aminooxy group, a hydrazine group, a hydrazide group, a semicarbazide group, an azide group, or an alkyne group. See, e.g., U.S. Patent No. 7,632,924 for suitable non-naturally occurring amino acids. Inclusion of a non-naturally occurring amino acid can provide for linkage to a polymer, a second polypeptide, a scaffold, etc. For example, a subject antibody linked to a water-soluble polymer can be made by reacting a water-soluble polymer (e.g., PEG) that comprises a carbonyl group to an the subject antibody that comprises a non-naturally encoded amino acid that comprises an aminooxy, hydrazine, hydrazide or semicarbazide group. As another example, a subject antibody linked to a water-soluble polymer can be made by reacting a subject antibody that comprises an alkyne-containing amino acid with a water-soluble polymer (e.g., PEG) that comprises an azide moiety; in some embodiments, the azide or alkyne group is linked to the PEG molecule through an amide linkage. A "non-naturally encoded amino acid" refers to an amino acid that is not one of the 20 common amino acids or pyrolysine or selenocysteine. Other terms that may be used synonymously with the term "non-naturally encoded amino acid" are "non-natural amino acid," "unnatural amino acid," "non-naturally-occurring amino acid," and

variously hyphenated and non-hyphenated versions thereof. The term "non-naturally encoded amino acid" also includes, but is not limited to, amino acids that occur by modification (e.g. post-translational modifications) of a naturally encoded amino acid (including but not limited to, the 20 common amino acids or pyrolysine and selenocysteine) but are not themselves naturally incorporated into a growing polypeptide chain by the translation complex. Examples of such non-naturally-occurring amino acids include, but are not limited to, N-acetylglucosaminyl-L-serine, N-acetylglucosaminyl-L-threonine, and O –phosphotyrosine.

In some embodiments, a subject antibody is linked (e.g., covalently linked) to a polymer (e.g., a polymer other than a polypeptide). Suitable polymers include, e.g., biocompatible polymers, and water-soluble biocompatible polymers. Suitable polymers include synthetic polymers and naturally-occurring polymers. Suitable polymers include, e.g., substituted or unsubstituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymers or branched or unbranched polysaccharides, e.g. a homo- or hetero-polysaccharide. Suitable polymers include, e.g., ethylene vinyl alcohol copolymer (commonly known by the generic name EVOH or by the trade name EVAL); polybutylmethacrylate; poly(hydroxyvalerate); poly(L-lactic acid); polycaprolactone; poly(lactide-co-glycolide); poly(hydroxybutyrate); poly(hydroxybutyrate-co-valerate); polydioxanone; polyorthoester; polyanhydride; poly(glycolic acid); poly(D,L-lactic acid); poly(glycolic acid-co-trimethylene carbonate); polyphosphoester; polyphosphoester urethane; poly(amino acids); cyanoacrylates; poly(trimethylene carbonate); poly(iminocarbonate); copoly(ether-esters) (e.g., poly(ethylene oxide)-poly(lactic acid) (PEO/PLA) co-polymers); polyalkylene oxalates; polyphosphazenes; biomolecules, such as fibrin, fibrinogen, cellulose, starch, collagen and hyaluronic acid; polyurethanes; silicones; polyesters; polyolefins; polyisobutylene and ethylene-alphaolefin copolymers; acrylic polymers and copolymers; vinyl halide polymers and copolymers, such as polyvinyl chloride; polyvinyl ethers, such as polyvinyl methyl ether; polyvinylidene halides, such as polyvinylidene fluoride and polyvinylidene chloride; polyacrylonitrile; polyvinyl ketones; polyvinyl aromatics, such as polystyrene; polyvinyl esters, such as polyvinyl acetate; copolymers of vinyl monomers with each other and olefins, such as ethylene-methyl methacrylate copolymers, acrylonitrile-styrene copolymers, ABS resins, and ethylene-vinyl acetate copolymers; polyamides, such as Nylon 66 and polycaprolactam; alkyd resins; polycarbonates; polyoxymethylenes; polyimides; polyethers; epoxy resins; polyurethanes; rayon; rayon-triacetate; cellulose; cellulose acetate; cellulose butyrate; cellulose acetate butyrate; cellophane; cellulose nitrate; cellulose propionate; cellulose ethers; amorphous Teflon; poly(ethylene glycol); and carboxymethyl cellulose.

Suitable synthetic polymers include unsubstituted and substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol) poly(vinylalcohol), and derivatives thereof, e.g., substituted

poly(ethyleneglycol) such as methoxypoly(ethyleneglycol), and derivatives thereof. Suitable naturally-occurring polymers include, e.g., albumin, amylose, dextran, glycogen, and derivatives thereof.

Suitable polymers can have an average molecular weight in a range of from 500 Da to 50000 Da, e.g., from 5000 Da to 40000 Da, or from 25000 to 40000 Da. For example, in some embodiments, where a subject antibody comprises a poly(ethylene glycol) (PEG) or methoxypoly(ethyleneglycol) polymer, the PEG or methoxypoly(ethyleneglycol) polymer can have a molecular weight in a range of from about 0.5 kiloDaltons (kDa) to 1 kDa, from about 1 kDa to 5 kDa, from 5 kDa to 10 kDa, from 10 kDa to 25 kDa, from 25 kDa to 40 kDa, or from 40 kDa to 60 kDa.

As noted above, in some embodiments, a subject antibody is covalently linked to a PEG polymer. In some embodiments, a subject scFv multimer is covalently linked to a PEG polymer. See, e.g., Albrecht et al. (2006) *J. Immunol. Methods* 310:100. Methods and reagents suitable for PEGylation of a protein are well known in the art and may be found in, e.g., U.S. Pat. No. 5,849,860. PEG suitable for conjugation to a protein is generally soluble in water at room temperature, and has the general formula $R(O\text{-}CH_2\text{-}CH_2)_nO\text{-}R$, where R is hydrogen or a protective group such as an alkyl or an alkanol group, and where n is an integer from 1 to 1000. Where R is a protective group, it generally has from 1 to 8 carbons.

The PEG conjugated to the subject antibody can be linear. The PEG conjugated to the subject protein may also be branched. Branched PEG derivatives such as those described in U.S. Pat. No. 5,643,575, "star-PEG's" and multi-armed PEG's such as those described in Shearwater Polymers, Inc. catalog "Polyethylene Glycol Derivatives 1997-1998." Star PEGs are described in the art including, e.g., in U.S. Patent No. 6,046,305.

A subject antibody can be glycosylated, e.g., comprise a covalently linked carbohydrate or polysaccharide moiety. Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to an antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation

sites). Similarly, removal of glycosylation sites can be accomplished by amino acid alteration within the native glycosylation sites of an antibody.

A subject antibody will in some embodiments comprise a "radiopaque" label, e.g. a label that can be easily visualized using for example x-rays. Radiopaque materials are well known to those of skill in the art. The most common radiopaque materials include iodide, bromide or barium salts. Other radiopaque materials are also known and include, but are not limited to organic bismuth derivatives (see, e.g., U.S. Pat. No. 5,939,045), radiopaque multiurethanes (see U.S. Pat. No. 5,346,981), organobismuth composites (see, e.g., U.S. Pat. No. 5,256,334), radiopaque barium multimer complexes (see, e.g., U.S. Pat. No. 4,866,132), and the like.

A subject antibody can be covalently linked to a second moiety (e.g., a lipid, a polypeptide other than a subject antibody, a synthetic polymer, a carbohydrate, and the like) using for example, glutaraldehyde, a homobifunctional cross-linker, or a heterobifunctional cross-linker. Glutaraldehyde cross-links polypeptides via their amino moieties. Homobifunctional cross-linkers (e.g., a homobifunctional imidoester, a homobifunctional N-hydroxysuccinimidyl (NHS) ester, or a homobifunctional sulfhydryl reactive cross-linker) contain two or more identical reactive moieties and can be used in a one step reaction procedure in which the cross-linker is added to a solution containing a mixture of the polypeptides to be linked. Homobifunctional NHS ester and imido esters cross-link amine containing polypeptides. In a mild alkaline pH, imido esters react only with primary amines to form imidoamides, and overall charge of the cross-linked polypeptides is not affected. Homobifunctional sulfhydryl reactive cross-linkers includes bismaleimidhexane (BMH), 1,5-difluoro-2,4-dinitrobenzene (DFDNB), and 1,4-di-(3',2'-pyridyldithio) propinoamido butane (DPDPB).

Heterobifunctional cross-linkers have two or more different reactive moieties (e.g., amine reactive moiety and a sulfhydryl-reactive moiety) and are cross-linked with one of the polypeptides via the amine or sulfhydryl reactive moiety, then reacted with the other polypeptide via the non-reacted moiety. Multiple heterobifunctional haloacetyl cross-linkers are available, as are pyridyl disulfide cross-linkers. Carbodiimides are a classic example of heterobifunctional cross-linking reagents for coupling carboxyls to amines, which results in an amide bond.

A subject antibody can be immobilized on a solid support. Suitable supports are well known in the art and comprise, inter alia, commercially available column materials, polystyrene beads, latex beads, magnetic beads, colloid metal particles, glass and/or silicon chips and surfaces, nitrocellulose strips, nylon membranes, sheets, duracytes, wells of reaction trays (e.g., multi-well plates), plastic tubes, etc. A solid support can comprise any of a variety of substances, including, e.g., glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amylose, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. Suitable methods for immobilizing a subject antibody onto a solid support are well known and include, but are not limited to ionic, hydrophobic,

covalent interactions and the like. Solid supports can be soluble or insoluble, e.g., in aqueous solution. In some embodiments, a suitable solid support is generally insoluble in an aqueous solution.

A subject antibody will in some embodiments comprise a detectable label. Suitable detectable labels include any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Suitable include, but are not limited to, magnetic beads (e.g. Dynabeads™), fluorescent dyes (e.g., fluorescein isothiocyanate, texas red, rhodamine, a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, and the like), radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase, luciferase, and others commonly used in an enzyme-linked immunosorbent assay (ELISA)), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g. polystyrene, polypropylene, latex, etc.) beads.

In some embodiments, a subject antibody comprises a contrast agent or a radioisotope, where the contrast agent or radioisotope is one that is suitable for use in imaging, e.g., imaging procedures carried out on humans. Non-limiting examples of labels include radioisotope such as ^{1231}I (iodine), ^{18}F (fluorine), ^{99}Tc (technetium), ^{111}In (indium), and ^{67}Ga (gallium), and contrast agent such as gadolinium (Gd), dysprosium, and iron. Radioactive Gd isotopes (^{153}Gd) also are available and suitable for imaging procedures in non-human mammals. A subject antibody can be labeled using standard techniques. For example, a subject antibody can be iodinated using chloramine T or 1,3,4,6-tetrachloro-3α,6α-dephenylglycouril. For fluorination, fluorine is added to a subject antibody during the synthesis by a fluoride ion displacement reaction. See, Muller-Gartner, H., TIB Tech., 16:122-130 (1998) and Saji, H., Crit. Rev. Ther. Drug Carrier Syst., 16(2):209-244 (1999) for a review of synthesis of proteins with such radioisotopes. A subject antibody can also be labeled with a contrast agent through standard techniques. For example, a subject antibody can be labeled with Gd by conjugating low molecular Gd chelates such as Gd diethylene triamine pentaacetic acid (GdDTPA) or Gd tetraazacyclododecanetetraacetic (GdDOTA) to the antibody. See, Caravan et al., Chem. Rev. 99:2293-2352 (1999) and Lauffer et al., J. Magn. Reson. Imaging, 3:11-16 (1985). A subject antibody can be labeled with Gd by, for example, conjugating polylysine-Gd chelates to the antibody. See, for example, Curtet et al., Invest. Radiol., 33(10):752-761 (1998). Alternatively, a subject antibody can be labeled with Gd by incubating paramagnetic polymerized liposomes that include Gd chelator lipid with avidin and biotinylated antibody. See, for example, Sipkins et al., Nature Med., 4:623-626 (1998).

Suitable fluorescent proteins that can be linked to a subject antibody include, but are not limited to, a green fluorescent protein from *Aequoria victoria* or a mutant or derivative thereof e.g., as described in U.S. Patent No. 6,066,476; 6,020,192; 5,985,577; 5,976,796; 5,968,750; 5,968,738; 5,958,713; 5,919,445; 5,874,304; e.g., Enhanced GFP, many such GFP which are available commercially, e.g., from Clontech, Inc.; a red fluorescent protein; a yellow fluorescent protein; any of a

variety of fluorescent and colored proteins from Anthozoan species, as described in, e.g., Matz et al. (1999) *Nature Biotechnol.* 17:969-973; and the like.

A subject antibody will in some embodiments be linked to (e.g., covalently or non-covalently linked) a fusion partner, e.g., a ligand; an epitope tag; a peptide; a protein other than an antibody; and the like. Suitable fusion partners include peptides and polypeptides that confer enhanced stability *in vivo* (e.g., enhanced serum half-life); provide ease of purification, e.g., $(His)_n$, e.g., 6His, and the like; provide for secretion of the fusion protein from a cell; provide an epitope tag, e.g., GST, hemagglutinin (HA; e.g., CYPYDVPDYA; SEQ ID NO: 168), FLAG (e.g., DYKDDDDK; SEQ ID NO:169), c-myc (e.g., CEQKLISEEDL; SEQ ID NO:170), and the like; provide a detectable signal, e.g., an enzyme that generates a detectable product (e.g., β-galactosidase, luciferase), or a protein that is itself detectable, e.g., a green fluorescent protein, a red fluorescent protein, a yellow fluorescent protein, etc.; provides for multimerization, e.g., a multimerization domain such as an Fc portion of an immunoglobulin; and the like.

The fusion may also include an affinity domain, including peptide sequences that can interact with a binding partner, e.g., such as one immobilized on a solid support, useful for identification or purification. Consecutive single amino acids, such as histidine, when fused to a protein, can be used for one-step purification of the fusion protein by high affinity binding to a resin column, such as nickel sepharose. Exemplary affinity domains include His5 (HHHHH) (SEQ ID NO: 171), HisX6 (HHHHHH) (SEQ ID NO: 172), C-myc (EQKLISEEDL) (SEQ ID NO: 173), Flag (DYKDDDDK) (SEQ ID NO: 174), StrepTag (WSHPQFEK) (SEQ ID NO: 175), hemagglutinin, e.g., HA Tag (YPYDVPDYA; SEQ ID NO: 176), glutathinone-S-transferase (GST), thioredoxin, cellulose binding domain, RYIRS (SEQ ID NO: 177), Phe-His-His-Thr (SEQ ID NO: 178), chitin binding domain, S-peptide, T7 peptide, SH2 domain, C-end RNA tag, WEAAAREACCRECCARA (SEQ ID NO: 179), metal binding domains, e.g., zinc binding domains or calcium binding domains such as those from calcium-binding proteins, e.g., calmodulin, troponin C, calcineurin B, myosin light chain, recoverin, S-modulin, visinin, VILIP, neurocalcin, hippocalcin, frequenin, caltractin, calpain large-subunit, S100 proteins, parvalbumin, calbindin D9K, calbindin D28K, and calretinin, inteins, biotin, streptavidin, MyoD, leucine zipper sequences, and maltose binding protein.

A subject antibody will in some embodiments be fused to a polypeptide that binds to an endogenous blood brain barrier (BBB) receptor. Linking a subject antibody to a polypeptide that binds to an endogenous BBB receptor facilitates crossing the BBB, e.g., in a subject treatment method (see below) involving administration of a subject antibody to an individual in need thereof. Suitable polypeptides that bind to an endogenous BBB include antibodies, e.g., monoclonal antibodies, or antigen-binding fragments thereof, that specifically bind to an endogenous BBB receptor. Suitable endogenous BBB receptors include, but are not limited to, an insulin receptor, a transferrin receptor, a

leptin receptor, a lipoprotein receptor, and an insulin-like growth factor receptor. See, e.g., U.S. Patent Publication No. 2009/0156498.

In some embodiments, a subject antibody comprises a polyamine modification. Polyamine modification of a subject antibody enhances permeability of the modified antibody at the BBB. A subject antibody can be modified with polyamines that are either naturally occurring or synthetic. See, for example, U.S. Pat. No. 5,670,477. Useful naturally occurring polyamines include putrescine, spermidine, spermine, 1,3-deaminopropane, norspermidine, syn-homospermidine, thermine, thermospermine, caldopentamine, homocaldopentamine, and canavalmine. Putrescine, spermidine and spermine are particularly useful. Synthetic polyamines are composed of the empirical formula $C_XH_YN_Z$, can be cyclic or acyclic, branched or unbranched, hydrocarbon chains of 3-12 carbon atoms that further include 1-6 NR or $N(R)_2$ moieties, wherein R is H, (C_1-C_4) alkyl, phenyl, or benzyl. Polyamines can be linked to an antibody using any standard crosslinking method.

In some embodiments, a subject antibody is modified to include a carbohydrate moiety, where the carbohydrate moiety can be covalently linked to the antibody. In some embodiments, a subject antibody is modified to include a lipid moiety, where the lipid moiety can be covalently linked to the antibody. Suitable lipid moieties include, e.g., an N-fatty acyl group such as N-lauroyl, N-oleoyl, etc.; a fatty amine such as dodecyl amine, oleoyl amine, etc.; a C3-C16 long-chain aliphatic lipid; and the like. See, e.g., U.S. Pat. No. 6,638,513). In some embodiments, a subject antibody is incorporated into a liposome.

Methods of producing a subject antibody

A subject antibody can be produced by any known method, e.g., conventional synthetic methods for protein synthesis; recombinant DNA methods; etc.

Where a subject antibody is a single chain polypeptide, it can be synthesized using standard chemical peptide synthesis techniques. Where a polypeptide is chemically synthesized, the synthesis may proceed via liquid-phase or solid-phase. Solid phase polypeptide synthesis (SPPS), in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence, is an example of a suitable method for the chemical synthesis of a subject antibody. Various forms of SPPS, such as Fmoc and Boc, are available for synthesizing a subject antibody. Techniques for solid phase synthesis are described by Barany and Merrifield, Solid-Phase Peptide Synthesis; pp. 3-284 in The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A., Merrifield, et al. J. Am. Chem. Soc., 85: 2149-2156 (1963); Stewart et al., Solid Phase Peptide Synthesis, 2nd ed. Pierce Chem. Co., Rockford, Ill. (1984); and Ganesan A. 2006 *Mini Rev. Med Chem.* 6:3-10 and Camarero JA et al. 2005 *Protein Pept Lett.* 12:723-8. Briefly, small insoluble, porous beads are treated with functional units on which peptide chains are built. After repeated cycling of coupling/deprotection, the free N-terminal amine of a solid-phase

attached is coupled to a single N-protected amino acid unit. This unit is then deprotected, revealing a new N-terminal amine to which a further amino acid may be attached. The peptide remains immobilized on the solid-phase and undergoes a filtration process before being cleaved off.

Standard recombinant methods can be used for production of a subject antibody. For example, nucleic acids encoding light and heavy chain variable regions, optionally linked to constant regions, are inserted into expression vectors. The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Expression control sequences include, but are not limited to, promoters (e.g., naturally-associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences. The expression control sequences can be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells (e.g., COS or CHO cells). Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the antibodies.

Because of the degeneracy of the code, a variety of nucleic acid sequences can encode each immunoglobulin amino acid sequence. The desired nucleic acid sequences can be produced by de novo solid-phase DNA synthesis or by polymerase chain reaction (PCR) mutagenesis of an earlier prepared variant of the desired polynucleotide. Oligonucleotide-mediated mutagenesis is an example of a suitable method for preparing substitution, deletion and insertion variants of target polypeptide DNA. See Adelman et al., DNA 2:183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a single-stranded DNA template. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that incorporates the oligonucleotide primer, and encodes the selected alteration in the target polypeptide DNA.

Suitable expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers (e.g., ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA sequences.

Escherichia coli is an example of a prokaryotic host cell that can be used for cloning a subject antibody-encoding polynucleotide. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as Salmonella, Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The

promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, are also useful for expression. Saccharomyces (e.g., *S. cerevisiae*) and Pichia are examples of suitable yeast host cells, with suitable vectors having expression control sequences (e.g., promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

In addition to microorganisms, mammalian cells (e.g., mammalian cells grown in *in vitro* cell culture) can also be used to express and produce the polypeptides of the present invention (e.g., polynucleotides encoding immunoglobulins or fragments thereof). See Winnacker, From Genes to Clones, VCH Publishers, N.Y., N.Y. (1987). Suitable mammalian host cells include CHO cell lines, various Cos cell lines, HeLa cells, myeloma cell lines, and transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (Queen et al., Immunol. Rev. 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Examples of suitable expression control sequences are promoters derived from immunoglobulin genes, SV40, adenovirus, bovine papilloma virus, cytomegalovirus and the like. See Co et al., J. Immunol. 148:1149 (1992).

Once synthesized (either chemically or recombinantly), the whole antibodies, their dimers, individual light and heavy chains, or other forms of a subject antibody (e.g., scFv, etc.) can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, high performance liquid chromatography (HPLC) purification, gel electrophoresis, and the like (see generally Scopes, Protein Purification (Springer-Verlag, N.Y., (1982)). A subject antibody can be substantially pure, e.g., at least about 80% to 85% pure, at least about 85% to 90% pure, at least about 90% to 95% pure, or 98% to 99%, or more, pure, e.g., free from contaminants such as cell debris, macromolecules other than a subject antibody, etc.

Compositions

The present disclosure provides a composition comprising a subject antibody. A subject antibody composition can comprise, in addition to a subject antibody, one or more of: a salt, e.g., NaCl, $MgCl_2$, KCl, $MgSO_4$, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; glycerol; and the like.

Nucleic Acids

The present disclosure provides nucleic acids comprising nucleotide sequences encoding a subject antibody. A nucleotide sequence encoding a subject antibody can be operably linked to one or more regulatory elements, such as a promoter and enhancer, that allow expression of the nucleotide sequence in the intended target cells (e.g., a cell that is genetically modified to synthesize the encoded antibody).

Suitable promoter and enhancer elements are known in the art. For expression in a bacterial cell, suitable promoters include, but are not limited to, lacI, lacZ, T3, T7, gpt, lambda P and trc. For expression in a eukaryotic cell, suitable promoters include, but are not limited to, light and/or heavy chain immunoglobulin gene promoter and enhancer elements; cytomegalovirus immediate early promoter; herpes simplex virus thymidine kinase promoter; early and late SV40 promoters; promoter present in long terminal repeats from a retrovirus; mouse metallothionein-I promoter; and various art-known tissue specific promoters.

In some embodiments, e.g., for expression in a yeast cell, a suitable promoter is a constitutive promoter such as an ADH1 promoter, a PGK1 promoter, an ENO promoter, a PYK1 promoter and the like; or a regulatable promoter such as a GAL1 promoter, a GAL10 promoter, an ADH2 promoter, a PHO5 promoter, a CUP1 promoter, a GAL7 promoter, a MET25 promoter, a MET3 promoter, a CYC1 promoter, a HIS3 promoter, an ADH1 promoter, a PGK promoter, a GAPDH promoter, an ADC1 promoter, a TRP1 promoter, a URA3 promoter, a LEU2 promoter, an ENO promoter, a TP1 promoter, and AOX1 (e.g., for use in *Pichia*). Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

Suitable promoters for use in prokaryotic host cells include, but are not limited to, a bacteriophage T7 RNA polymerase promoter; a trp promoter; a lac operon promoter; a hybrid promoter, e.g., a lac/tac hybrid promoter, a tac/trc hybrid promoter, a trp/lac promoter, a T7/lac promoter; a trc promoter; a tac promoter, and the like; an araBAD promoter; *in vivo* regulated promoters, such as an *ssaG* promoter or a related promoter (*see*, e.g., U.S. Patent Publication No. 20040131637), a *pagC* promoter (Pulkkinen and Miller, *J. Bacteriol.*, 1991: 173(1): 86-93; Alpuche-Aranda et al., PNAS, 1992; 89(21): 10079-83), a *nirB* promoter (Harborne et al. (1992) *Mol. Micro.* 6:2805-2813), and the like (*see*, e.g., Dunstan et al. (1999) *Infect. Immun.* 67:5133-5141; McKelvie et al. (2004) *Vaccine* 22:3243-3255; and Chatfield et al. (1992) *Biotechnol.* 10:888-892); a sigma70 promoter, e.g., a consensus sigma70 promoter (see, e.g., GenBank Accession Nos. AX798980, AX798961, and AX798183); a stationary phase promoter, e.g., a *dps* promoter, an *spv* promoter, and the like; a promoter derived from the pathogenicity island SPI-2 (*see*, e.g., WO96/17951); an actA promoter (*see*, e.g., Shetron-Rama et al. (2002) *Infect. Immun.* 70:1087-1096); an rpsM promoter (*see*, e.g., Valdivia and Falkow (1996). *Mol. Microbiol.* 22:367); a tet promoter (*see*, e.g., Hillen,W. and Wissmann,A. (1989) In Saenger,W. and

Heinemann,U. (eds), *Topics in Molecular and Structural Biology, Protein–Nucleic Acid Interaction.* Macmillan, London, UK, Vol. 10, pp. 143–162); an SP6 promoter (*see*, e.g., Melton et al. (1984) *Nucl. Acids Res.* 12:7035); and the like. Suitable strong promoters for use in prokaryotes such as *Escherichia coli* include, but are not limited to Trc, Tac, T5, T7, and $P_{Lambda.}$ Non-limiting examples of operators for use in bacterial host cells include a lactose promoter operator (LacI repressor protein changes conformation when contacted with lactose, thereby preventing the LacI repressor protein from binding to the operator), a tryptophan promoter operator (when complexed with tryptophan, TrpR repressor protein has a conformation that binds the operator; in the absence of tryptophan, the TrpR repressor protein has a conformation that does not bind to the operator), and a tac promoter operator (see, for example, deBoer et al. (1983) Proc. Natl. Acad. Sci. U.S.A. 80:21-25).

A nucleotide sequence encoding a subject antibody can be present in an expression vector and/or a cloning vector. Where a subject antibody comprises two separate polypeptides, nucleotide sequences encoding the two polypeptides can be cloned in the same or separate vectors. An expression vector can include a selectable marker, an origin of replication, and other features that provide for replication and/or maintenance of the vector.

Large numbers of suitable vectors and promoters are known to those of skill in the art; many are commercially available for generating a subject recombinant constructs. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia).

Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous proteins. A selectable marker operative in the expression host may be present. Suitable expression vectors include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., Invest Opthalmol Vis Sci 35:2543 2549, 1994; Borras et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, e.g., Ali et al., Hum Gene Ther 9:81 86, 1998, Flannery et al., PNAS 94:6916 6921, 1997; Bennett et al., Invest Opthalmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997, Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:10319 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and

vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

As noted above, a subject nucleic acid comprises a nucleotide sequence encoding a subject antibody. A subject nucleic acid can comprise a nucleotide sequence encoding heavy- and light-chain CDRs. In some embodiments, a subject nucleic acid comprises a nucleotide sequence encoding heavy- and light-chain CDRs, where the CDR-encoding sequences are interspersed with FR-encoding nucleotide sequences. In some embodiments, the FR-encoding nucleotide sequences are human FR-encoding nucleotide sequences.

Cells

The present disclosure provides isolated genetically modified host cells (e.g., *in vitro* cells) that are genetically modified with a subject nucleic acid. In some embodiments, a subject isolated genetically modified host cell can produce a subject antibody.

Suitable host cells include eukaryotic host cells, such as a mammalian cell, an insect host cell, a yeast cell; and prokaryotic cells, such as a bacterial cell. Introduction of a subject nucleic acid into the host cell can be effected, for example by calcium phosphate precipitation, DEAE dextran mediated transfection, liposome-mediated transfection, electroporation, or other known method.

Suitable mammalian cells include primary cells and immortalized cell lines. Suitable mammalian cell lines include human cell lines, non-human primate cell lines, rodent (e.g., mouse, rat) cell lines, and the like. Suitable mammalian cell lines include, but are not limited to, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCLI.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

Suitable yeast cells include, but are not limited to, Pichia pastoris, Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stiptis, Pichia methanolica, Pichia sp., Saccharomyces cerevisiae, Saccharomyces sp., Hansenula polymorpha, Kluyveromyces sp., Kluyveromyces lactis, Candida albicans, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Trichoderma reesei, Chrysosporium lucknowense, Fusarium sp., Fusarium gramineum, Fusarium venenatum, Neurospora crassa, Chlamydomonas reinhardtii, and the like.

Suitable prokaryotic cells include, but are not limited to, any of a variety of laboratory strains of *Escherichia coli*, *Lactobacillus* sp., *Salmonella* sp., *Shigella* sp., and the like. See, e.g., Carrier et al. (1992) *J. Immunol.* 148:1176-1181; U.S. Patent No. 6,447,784; and Sizemore et al. (1995) *Science*

270:299-302. Examples of Salmonella strains which can be employed in the present invention include, but are not limited to, *Salmonella typhi* and *S. typhimurium*. Suitable Shigella strains include, but are not limited to, *Shigella flexneri*, *Shigella sonnei*, and *Shigella disenteriae*. Typically, the laboratory strain is one that is non-pathogenic. Non-limiting examples of other suitable bacteria include, but are not limited to, *Bacillus subtilis*, *Pseudomonas pudita*, *Pseudomonas aeruginosa*, *Pseudomonas mevalonii*, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Rhodococcus* sp., and the like. In some embodiments, the host cell is *Escherichia coli*.

Formulations and Pharmaceutical Compositions

The present disclosure provides compositions, including pharmaceutical compositions, comprising a subject antibody. In general, a formulation comprises an effective amount of a subject antibody. An "effective amount" means a dosage sufficient to produce a desired result, e.g., reduction in weight or blood glucose. Generally, the desired result is at least a reduction in a symptom of a BDNF-associated disorder or condition as compared to a control. A subject antibody can be delivered in such a manner as to avoid the blood-brain barrier, as described in more detail below. A subject antibody can be formulated and/or modified to enable the antibody to cross the blood-brain barrier, if necessary. A treatment involves administering treatment to a patient already suffering from a disease thus causing a therapeutically beneficial effect, such as ameliorating existing symptoms, ameliorating the underlying causes of symptoms, postponing or preventing the further development of a disorder, and/or reducing the severity of symptoms that will or are expected to develop.

Formulations

In the subject methods, a subject antibody can be administered to the host using any convenient means capable of resulting in the desired therapeutic effect or diagnostic effect. Thus, the agent can be incorporated into a variety of formulations for therapeutic administration. More particularly, a subject antibody can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

In pharmaceutical dosage forms, a subject antibody can be administered in the form of their pharmaceutically acceptable salts, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

For oral preparations, a subject antibody can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or

sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

A subject antibody can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

Pharmaceutical compositions comprising a subject antibody are prepared by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients, stabilizers, surfactants, buffers and/or tonicity agents. Acceptable carriers, excipients and/or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid, glutathione, cysteine, methionine and citric acid; preservatives (such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, or combinations thereof); amino acids such as arginine, glycine, ornithine, lysine, histidine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophan, methionine, serine, proline and combinations thereof; monosaccharides, disaccharides and other carbohydrates; low molecular weight (less than about 10 residues) polypeptides; proteins, such as gelatin or serum albumin; chelating agents such as EDTA; sugars such as trehalose, sucrose, lactose, glucose, mannose, maltose, galactose, fructose, sorbose, raffinose, glucosamine, N-methylglucosamine, galactosamine, and neuraminic acid; and/or non-ionic surfactants such as Tween, Brij Pluronics, Triton-X, or polyethylene glycol (PEG).

The pharmaceutical composition may be in a liquid form, a lyophilized form or a liquid form reconstituted from a lyophilized form, wherein the lyophilized preparation is to be reconstituted with a sterile solution prior to administration. The standard procedure for reconstituting a lyophilized composition is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization); however solutions comprising antibacterial agents may be used for the production of pharmaceutical compositions for parenteral administration; see also Chen (1992) Drug Dev Ind Pharm 18, 1311-54.

Exemplary antibody concentrations in a subject pharmaceutical composition may range from about 1 mg/mL to about 200 mg/ml or from about 50 mg/mL to about 200 mg/mL, or from about 150 mg/mL to about 200 mg/mL.

An aqueous formulation of the antibody may be prepared in a pH-buffered solution, e.g., at pH ranging from about 4.0 to about 7.0, or from about 5.0 to about 6.0, or alternatively about 5.5. Examples of buffers that are suitable for a pH within this range include phosphate-, histidine-, citrate-, succinate-, acetate-buffers and other organic acid buffers. The buffer concentration can be from about 1 mM to

about 100 mM, or from about 5 mM to about 50 mM, depending, e.g., on the buffer and the desired tonicity of the formulation.

A tonicity agent may be included in the antibody formulation to modulate the tonicity of the formulation. Exemplary tonicity agents include sodium chloride, potassium chloride, glycerin and any component from the group of amino acids, sugars as well as combinations thereof. In some embodiments, the aqueous formulation is isotonic, although hypertonic or hypotonic solutions may be suitable. The term "isotonic" denotes a solution having the same tonicity as some other solution with which it is compared, such as physiological salt solution or serum. Tonicity agents may be used in an amount of about 5 mM to about 350 mM, e.g., in an amount of 100 mM to 350 nM.

A surfactant may also be added to the antibody formulation to reduce aggregation of the formulated antibody and/or minimize the formation of particulates in the formulation and/or reduce adsorption. Exemplary surfactants include polyoxyethylensorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers (Brij), alkylphenylpolyoxyethylene ethers (Triton-X), polyoxyethylene-polyoxypropylene copolymer (Poloxamer, Pluronic), and sodium dodecyl sulfate (SDS). Examples of suitable polyoxyethylenesorbitan-fatty acid esters are polysorbate 20, (sold under the trademark Tween 20™) and polysorbate 80 (sold under the trademark Tween 80™). Examples of suitable polyethylene-polypropylene copolymers are those sold under the names Pluronic® F68 or Poloxamer 188™. Examples of suitable Polyoxyethylene alkyl ethers are those sold under the trademark Brij™. Exemplary concentrations of surfactant may range from about 0.001% to about 1% w/v.

A lyoprotectant may also be added in order to protect the labile active ingredient (e.g. a protein) against destabilizing conditions during the lyophilization process. For example, known lyoprotectants include sugars (including glucose and sucrose); polyols (including mannitol, sorbitol and glycerol); and amino acids (including alanine, glycine and glutamic acid). Lyoprotectants can be included in an amount of about 10 mM to 500 nM.

In some embodiments, a subject formulation includes a subject antibody, and one or more of the above-identified agents (e.g., a surfactant, a buffer, a stabilizer, a tonicity agent) and is essentially free of one or more preservatives, such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, and combinations thereof. In other embodiments, a preservative is included in the formulation, e.g., at concentrations ranging from about 0.001 to about 2% (w/v).

For example, a subject formulation can be a liquid or lyophilized formulation suitable for parenteral administration, and can comprise: about 1 mg/mL to about 200 mg/mL of a subject antibody; about 0.001 % to about 1 % of at least one surfactant; about 1 mM to about 100 mM of a buffer; optionally about 10 mM to about 500 mM of a stabilizer; and about 5 mM to about 305 mM of a tonicity agent; and has a pH of about 4.0 to about 7.0.

As another example, a subject parenteral formulation is a liquid or lyophilized formulation comprising: about 1 mg/mL to about 200 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM Sucrose; and has a pH of 5.5.

As another example, a subject parenteral formulation comprises a lyophilized formulation comprising: 1) 15 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM sucrose; and has a pH of 5.5; or 2) 75 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM sucrose; and has a pH of 5.5;or 3) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM Sucrose; and has a pH of 5.5; or 4) 75 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 6) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5.

As another example, a subject parenteral formulation is a liquid formulation comprising:1) 7.5 mg/mL of a subject antibody; 0.022% Tween 20 w/v; 120 mM L-histidine; and 250 125 mM sucrose; and has a pH of 5.5; or 2) 37.5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 10 mM L-histidine; and 125 mM sucrose; and has a pH of 5.5; or 3) 37.5 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 10 mM L-histidine; and 125 mM sucrose; and has a pH of 5.5; or 4) 37.5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 10 mM L-histidine; 125 mM trehalose; and has a pH of 5.5; or 5) 37.5 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 10 mM L-histidine; and 125 mM trehalose; and has a pH of 5.5; or 6) 5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 7) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM mannitol; and has a pH of 5.5; or 8) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L histidine; and 140 mM sodium chloride; and has a pH of 5.5;or 9) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 10) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM mannitol; and has a pH of 5.5; or 11) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 140 mM sodium chloride; and has a pH of 5.5; or 12) 10 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 20 mM L-histidine; and 40 mM sodium chloride; and has a pH of 5.5.

A subject antibody can be utilized in aerosol formulation to be administered via inhalation. A subject antibody can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

Furthermore, a subject antibody can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. A subject antibody can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may comprise a subject antibody in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for a subject antibody may depend on the particular antibody employed and the effect to be achieved, and the pharmacodynamics associated with each antibody in the host.

Other modes of administration will also find use with the subject invention. For instance, a subject antibody can be formulated in suppositories and, in some cases, aerosol and intranasal compositions. For suppositories, the vehicle composition will include traditional binders and carriers such as, polyalkylene glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), e.g., about 1% to about 2%.

Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

A subject antibody can be administered as an injectable formulation. Typically, injectable compositions are prepared as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the antibody encapsulated in liposome vehicles.

Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of a subject antibody adequate to achieve the desired state in the subject being treated.

The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH

adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

In some embodiments, a subject antibody is formulated in a controlled release formulation. Sustained-release preparations may be prepared using methods well known in the art. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody in which the matrices are in the form of shaped articles, e.g. films or microcapsules. Examples of sustained-release matrices include polyesters, copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, hydrogels, polylactides, degradable lactic acid-glycolic acid copolymers and poly-D-(-)-3-hydroxybutyric acid. Possible loss of biological activity and possible changes in immunogenicity of antibodies comprised in sustained-release preparations may be prevented by using appropriate additives, by controlling moisture content and by developing specific polymer matrix compositions.

Controlled release within the scope of this invention can be taken to mean any one of a number of extended release dosage forms. The following terms may be considered to be substantially equivalent to controlled release, for the purposes of the present invention: continuous release, controlled release, delayed release, depot, gradual release, long-term release, programmed release, prolonged release, proportionate release, protracted release, repository, retard, slow release, spaced release, sustained release, time coat, timed release, delayed action, extended action, layered-time action, long acting, prolonged action, repeated action, slowing acting, sustained action, sustained-action medications, and extended release. Further discussions of these terms may be found in Lesczek Krowczynski, Extended-Release Dosage Forms, 1987 (CRC Press, Inc.).

The various controlled release technologies cover a very broad spectrum of drug dosage forms. Controlled release technologies include, but are not limited to physical systems and chemical systems.

Physical systems include, but are not limited to, reservoir systems with rate-controlling membranes, such as microencapsulation, macroencapsulation, and membrane systems; reservoir systems without rate-controlling membranes, such as hollow fibers, ultra microporous cellulose triacetate, and porous polymeric substrates and foams; monolithic systems, including those systems physically dissolved in non-porous, polymeric, or elastomeric matrices (e.g., nonerodible, erodible, environmental agent ingression, and degradable), and materials physically dispersed in non-porous, polymeric, or elastomeric matrices (e.g., nonerodible, erodible, environmental agent ingression, and degradable); laminated structures, including reservoir layers chemically similar or dissimilar to outer control layers; and other physical methods, such as osmotic pumps, or adsorption onto ion-exchange resins.

Chemical systems include, but are not limited to, chemical erosion of polymer matrices (e.g., heterogeneous, or homogeneous erosion), or biological erosion of a polymer matrix (e.g., heterogeneous, or homogeneous). Additional discussion of categories of systems for controlled release may be found in

Agis F. Kydonieus, Controlled Release Technologies: Methods, Theory and Applications, 1980 (CRC Press, Inc.).

There are a number of controlled release drug formulations that are developed for oral administration. These include, but are not limited to, osmotic pressure-controlled gastrointestinal delivery systems; hydrodynamic pressure-controlled gastrointestinal delivery systems; membrane permeation-controlled gastrointestinal delivery systems, which include microporous membrane permeation-controlled gastrointestinal delivery devices; gastric fluid-resistant intestine targeted controlled-release gastrointestinal delivery devices; gel diffusion-controlled gastrointestinal delivery systems; and ion-exchange-controlled gastrointestinal delivery systems, which include cationic and anionic drugs. Additional information regarding controlled release drug delivery systems may be found in Yie W. Chien, Novel Drug Delivery Systems, 1992 (Marcel Dekker, Inc.). Some of these formulations are discussed herein.

Dosages

A suitable dosage can be determined by an attending physician or other qualified medical personnel, based on various clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex of the patient, time, and route of administration, general health, and other drugs being administered concurrently. A subject antibody may be administered in amounts between 1 ng/kg body weight and 20 mg/kg body weight per dose, e.g. between 0.1 mg/kg body weight to 10 mg/kg body weight, e.g. between 0.5 mg/kg body weight to 5 mg/kg body weight; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it can also be in the range of 1 μg to 10 mg per kilogram of body weight per minute.

Those of skill will readily appreciate that dose levels can vary as a function of the specific antibody, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

Routes of administration

A subject antibody is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

In some embodiments, the antibody can be administered to the patient as an intravenous infusion (200 mg or 2 mg/kg, up to 200 mg) over 10-60 (e.g., 30 minutes), every 2-4 (e.g., three) weeks in a pharmaceutically acceptable carrier, e.g., PBS.

Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, subcutaneous, intradermal, topical application, intravenous, intraarterial,

rectal, nasal, oral, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the antibody and/or the desired effect. A subject antibody composition can be administered in a single dose or in multiple doses. In some embodiments, a subject antibody composition is administered orally. In some embodiments, a subject antibody composition is administered via an inhalational route. In some embodiments, a subject antibody composition is administered intranasally. In some embodiments, a subject antibody composition is administered locally. In some embodiments, a subject antibody composition is administered intracranially. In some embodiments, a subject antibody composition is administered intravenously.

The agent can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of a subject antibody. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

A subject antibody can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

By treatment is meant at least an amelioration of the symptoms associated with the pathological condition afflicting the host, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, *e.g.* symptom, associated with the pathological condition being treated, such as BDNF-associated disorder or condition. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, *e.g.* prevented from happening, or stopped, *e.g.* terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

In some embodiments, a subject antibody is administered by injection and/or delivery, e.g., to a site in a brain artery or directly into brain tissue. A subject antibody can also be administered directly to a target site e.g., by biolistic delivery to the target site.

A variety of hosts (wherein the term "host" is used interchangeably herein with the terms "subject," "individual," and "patient") are treatable according to the subject methods. Generally such hosts are "mammals" or "mammalian," where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (*e.g.*, dogs and cats), rodentia (*e.g.*, mice,

guinea pigs, and rats), and primates (*e.g.,* humans, chimpanzees, and monkeys). In some embodiments, the hosts will be humans.

Kits with unit doses of a subject antibody, e.g. in oral or injectable doses, are provided. In such kits, in addition to the containers containing the unit doses will be an informational package insert describing the use and attendant benefits of the antibody in treating pathological condition of interest. Preferred compounds and unit doses are those described herein above.

Treatment Methods

As noted above, the present disclosure provides a method of treating a BDNF-associated disorder, by blocking or interfering with the biological activity of BDNF. In one embodiment, a therapeutically effective amount of a subject antibody is administered to a subject in need thereof. Methods of administration and delivery are also provided.

BDNF-associated disorders include, but are not limited to, disorders associated with aberrant synaptic transmission (which may be caused by, e.g., aberrant glutamatergic signaling, NMDA receptor activity, synapse stability, GABAergic signaling, synaptogenesis or dendritogenesis), aberrant neurogenesis or aberrant neurogenesis, or aberrant cognitive function. In particular embodiments the subject antibody may be used to treat as depression, schizophrenia, obsessive-compulsive disorder, Alzheimer's disease, Huntington's disease, Rett syndrome, dementia, anorexia nervosa, epilepsy or bulimia nervosa, which have all been linked to aberrant BDNF activity. In addition, BDNF is believed to be critical mediator of vulnerability to stress, memory of fear/trauma, and stress-related disorders such as post-traumatic stress disorder. BDNF has also been linked to obesity, drug addiction, psychological dependence, pain, and pathogenesis of post-chemotherapy cognitive impairment (PCCI, also known as chemo brain) and fatigue. Thus, the present antibody can be used to treat any of those disorders.

It is noted that a pharmaceutical composition comprising a subject antibody can be co-administered with another compound. The identity and properties of compound co-administered with the BDNF binding protein will depend on the nature of the condition to be treated or ameliorated. A non-limiting list of examples of compounds that can be administered in combination with a pharmaceutical composition comprising an anti-BDNF antibody include compound that modulates brain function.

Also provided are kits for practicing the disclosed methods. Such kits can comprise a pharmaceutical composition such as those described herein, including nucleic acids encoding the peptides or proteins provided herein, vectors and cells comprising such nucleic acids, and pharmaceutical compositions comprising such nucleic acid-containing compounds, which can be provided in a sterile container. Optionally, instructions on how to employ the provided pharmaceutical composition in the treatment of a BDNF-associated disorder can also be included or be made available to a patient or a medical service provider.

In one aspect, a kit comprises (a) a pharmaceutical composition comprising a therapeutically effective amount of a subject antibody; and (b) one or more containers for the pharmaceutical composition. Such a kit can also comprise instructions for the use thereof; the instructions can be tailored to the precise BDNF-associated disorder being treated. The instructions can describe the use and nature of the materials provided in the kit. In certain embodiments, kits include instructions for a patient to carry out administration to treat a BDNF-associated disorder.

Instructions can be printed on a substrate, such as paper or plastic, etc., and can be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (e.g., associated with the packaging), etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, such as over the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded.

Often it will be desirable that some or all components of a kit are packaged in suitable packaging to maintain sterility. The components of a kit can be packaged in a kit containment element to make a single, easily handled unit, where the kit containment element, e.g., box or analogous structure, may or may not be an airtight container, e.g., to further preserve the sterility of some or all of the components of the kit.

In some embodiments, an effective amount of a subject antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce an adverse symptom of a BDNF-associated disorder or condition by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the severity of the adverse symptom in the absence of treatment with the antibody.

Subjects Suitable for Treatment

A variety of subjects are suitable for treatment with a subject method. Suitable subjects include any individual, e.g., a human, who has a BDNF-associated disorder or condition, who has been diagnosed with a BDNF-associated disorder or condition, who is at risk for developing a BDNF-associated disorder or condition, who has had a BDNF-associated disorder or condition and is at risk for recurrence of the BDNF-associated disorder or condition, or who is recovering from a BDNF-associated disorder or condition.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

EXAMPLE 1

Anti-BDNF human monoclonal antibodies were generated using transgenic chickens (OmniChicken™) that express human antibody genes: human kappa light chain and human VH, with the chicken constant regions (Ching et al MAbs. 2018 10: 71–80).

Generation of anti-BDNF antibodies from primary chicken B cells

Five transgenic chickens (OmniChicken™) were immunized initially with 200ug of recombinant, human BDNF (Peprotech, Cat# 450-02) then boosted every 14 days with 100ug. One bird was boosted six times; two birds were boosted five times; one bird was boosted four times, and one bird was boosted three times. The serum immune response was monitored by ELISA on human his-tagged BDNF (R and D Systems, Cat# 248-BDB-010/CF).

Splenocytes were isolated and tested using the GEM assay as described in (Mettler Izquierdo et al Microscopy (Oxf). 2016 65: 341–352). Briefly, large beads (Life Technologies #A37306) were coated with a oligoclonal anti-his antibody (Life Technologies, Cat# 710286) for four hours followed by adsorption of his-tagged BDNF overnight. Beads were blocked in 3% milk in DPBS for 1h at room temperature then washed three times in DPBS. Beads were mixed with 2e7 primary splenocytes and a Alexa Fluor 594-conjugated goat anti-chicken IgY (Thermo Fisher Scientific #A-11042) for the detection. The mixture was plated and positive clones were detected under microscope using a CY3 filter. Cells secreting IgY that bound to the beads were selected.

Antibody cloning and binding confirmation

Positive clones were transferred into a 96-well plate containing a PCR mixture consisting of dNTP, primers and enzyme mix. The variable regions of heavy and light chains were cloned and assembled into a single chain Fv-Fc mammalian expression vector. Sanger sequencing was performed by GeneWiz and sequences were evaluated using DNASTAR Lasergene 15. Unique scFv-Fcs were

transiently expressed in Expi293 cells (Life Technologies, #A14527) grown in Expi293 Expression Medium (Life Technologies, #A1435101) after transfection using ExpiFectamine 293 transfection reagent and supplements (Life Technologies, #A14524). Cells were incubated for 3-5 days with shaking at 37°C in a 8% CO2 humidified incubator. Supernatants were harvested, quantitated using a Blitz Instrument (Pall ForteBio), and tested for binding activity by ELISA on plates coated with BDNF (Peprotech).

In the ELISA, EXPI293 cells were transfected with individual clones, and culture supernatants were collected 72 hours post transfection. For the ELISA assay, high-binding polystyrene plates were coated with human BDNF (PeproTech) at 2 ug/mL. Culture supernatants were added at 1 ug/mL followed by 5X serial dilutions down the plate. Plates were incubated for 1 hr at room temperature, washed and then incubated with anti-hu Fc-HRP at 0.5 ug/mL. Following a 1 hr incubation, plates were washed and developed with a colorimetric substrate indicator. The optical density at 280 nm was measured. The data shown below represent the reading at an antibody concentration of 1 ug/mL.

The sequences of the antibodies are shown in Figs.1 (HC) and 2 (LC). A phylogenetic tree of the antibodies is shown in Fig. 3. ELISA data for the antibodies is shown in the table below:

Clone	ELISA
40222p1.1.G4	2.673
40222p1.1.H1	2.456
40222p1.1.H9	2.818
40222p1.1.H10	2.611
40338p1.1.B11	2.06
40338p1.1.C12	2.066
40338p1.1.D11	2.037
40338p1.1.D12	1.656
40338p1.1.H8	2.155
40338p1.2.B4	1.953
40338p1.2.C5	1.986
40338p1.2.D7	1.132
40338p1.2.F2	2.162
40338p2.1.A7	2.506
40338p2.1.C12	1.87
40338p2.1.D4	1.971
40338p2.1.H12	2.764
40338p3.1.A5	2.358
40338p3.1.A9	2.642
40338p3.1.A10	2.454
40338p3.1.B6	2.438
40338p3.1.B12	2.608
40338p3.1.C4	2.307
40338p3.1.C7	2.495
40338p3.1.C11	2
40338p3.1.D7	0.799
40338p3.1.E7	1.295
40338p3.1.E12	2.606
40338p3.1.F4	2.658
40338p3.1.F6	2.618
40338p3.1.F10	2.731
40338p3.1.G2	2.934
40338p3.1.G5	2.692
40338p3.1.G10	2.623
40338p3.1.G12	2.65
40338p3.1.H6	2.53
40338p3.1.H7	0.472
40338p3.1.H10	2.716
40616p3.1.A10	2.124
40616p3.1.A11	1.529
40616p3.1.B3	2.043
40616p3.1.C2	1.26
40616p3.1.C7	1.617
40616p3.1.D3	1.339
40616p3.1.E8	1.675
40616p3.1.H5	2.172
40616p3.2.A6	1.598
40616p3.2.B4	2.34
40616p3.2.B8	1.996
40616p3.2.E12	1.824
40616p3.2.F7	2.276
40616p3.2.G9	2.122
40616p3.2.H8	2.307
40344p1.1.A7	2.683
40344p1.1.B4	2.737
40347p1.1.A4	2.311
40347p1.1.A6	0.467
40347p1.1.B12	1.876
40347p1.1.F9	1.166
40347p2.2.A2	2.607
40347p2.2.A9	2.665
40347p2.2.C8	2.06
40347p2.2.E2	2.8
40347p3.1.A10	1.434
40347p3.1.D12	1.354

40347p3.1.E1	2.584
40347p3.1.E11	2.68
40347p3.1.E9	1.514
40347p3.1.F3	2.672
40347p3.1.G5	2.013
40347p3.1.H12	3.051
40347p3.2.B10	3.091
40347p3.2.B12	2.999
40347p3.2.C2	1.217

40347p3.2.D9	0.513
40347p3.2.E4	1.73
40347p3.2.E5	2.209
40347p3.2.F1	2.409
40347p3.2.F11	2.318
40347p3.2.F4	2.603
40347p4.1.B6	3.557
40347p4.1.D6	1.768
40347p4.1.D7	2.155

40347p4.1.E4	2.664
40347p4.1.F7	2.651
40347p4.2.A4	3.128
40347p4.2.B4	2.197
40347p4.2.B11	1.039
40347p4.2.C9	2.937
40347p4.2.E8	2.891

While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. An antibody that binds to the human brain-derived neurotrophic factor (BDNF), wherein the antibody comprises:

(a) a variable domain comprising:

i. heavy chain CDR1, CDR2 and CDR3 regions that are identical to the heavy chain CDR1, CDR2 and CDR3 regions of an antibody selected from Fig. 1; and

ii. light chain CDR1, CDR2 and CDR3 regions that are identical to the light chain CDR1, CDR2 and CDR3 regions of the antibody, selected from Fig. 2; or

(b) a variant of said variable domain of (a) that is otherwise identical to said antibody variable domain except for up to 10 amino acid substitutions in the collective CDR regions of the variable domain of (a).

2. The antibody of claim 1, wherein the antibody comprises:

a heavy chain variable domain comprising an amino acid sequence that is at least 90% (e.g., at least 95%) identical to the amino acid sequence of the heavy chain variable domain of an antibody selected from Fig. 1; and

a light chain variable domain comprising an amino acid sequence that is at least 90% (e.g., at least 95%) identical to the light chain variable domain of the antibody, selected from Fig. 2.

3. The antibody of any prior claim, wherein the antibody inhibits BDNF signaling.

4. The antibody of any prior claim, wherein the heavy chain variable domain and the light chain variable domain are present in separate polypeptides.

5. The antibody of any prior claim, wherein the heavy chain variable domain and the light chain variable domain are present in a single polypeptide.

6. The antibody of any prior claim, wherein the antibody binds human BDNF with an affinity in the range of 10^7 M^{-1} to 10^{12} M^{-1}.

7. The antibody of any prior claim, wherein the antibody comprises a covalently linked non-peptide synthetic polymer.

8. The antibody of claim 7, wherein the synthetic polymer is poly(ethylene glycol) polymer.

9. The antibody of any prior claim, wherein the antibody comprises a covalently linked lipid or fatty acid moiety.

10. The antibody of any prior claim, wherein the antibody comprises a covalently linked polysaccharide or carbohydrate moiety.

11. The antibody of any prior claim, wherein the antibody is a single chain Fv (scFv) antibody.

12. The antibody of claim 10, wherein the scFv is multimerized.

13. A pharmaceutical composition comprising:
a) the antibody of any prior claim; and
b) a pharmaceutically acceptable carrier.

14. The pharmaceutical composition of claim 13, wherein the antibody is encapsulated in a liposome.

15. A method for inhibiting BDNF signaling, comprising contacting a cell comprising a TrkB, LNGFR or alpha-7 nicotinic receptor with BDNF in the presence of an antibody of any of claims 1-12.

16. A method of inhibiting BDNF signaling in a subject, comprising administering to the subject an effective amount of the antibody of any of claims 1-12.

17. A method of treating a BDNF-related disorder, comprising administering to a subject an effective amount of the antibody of any of any of claims 1-14.

FIG. 1

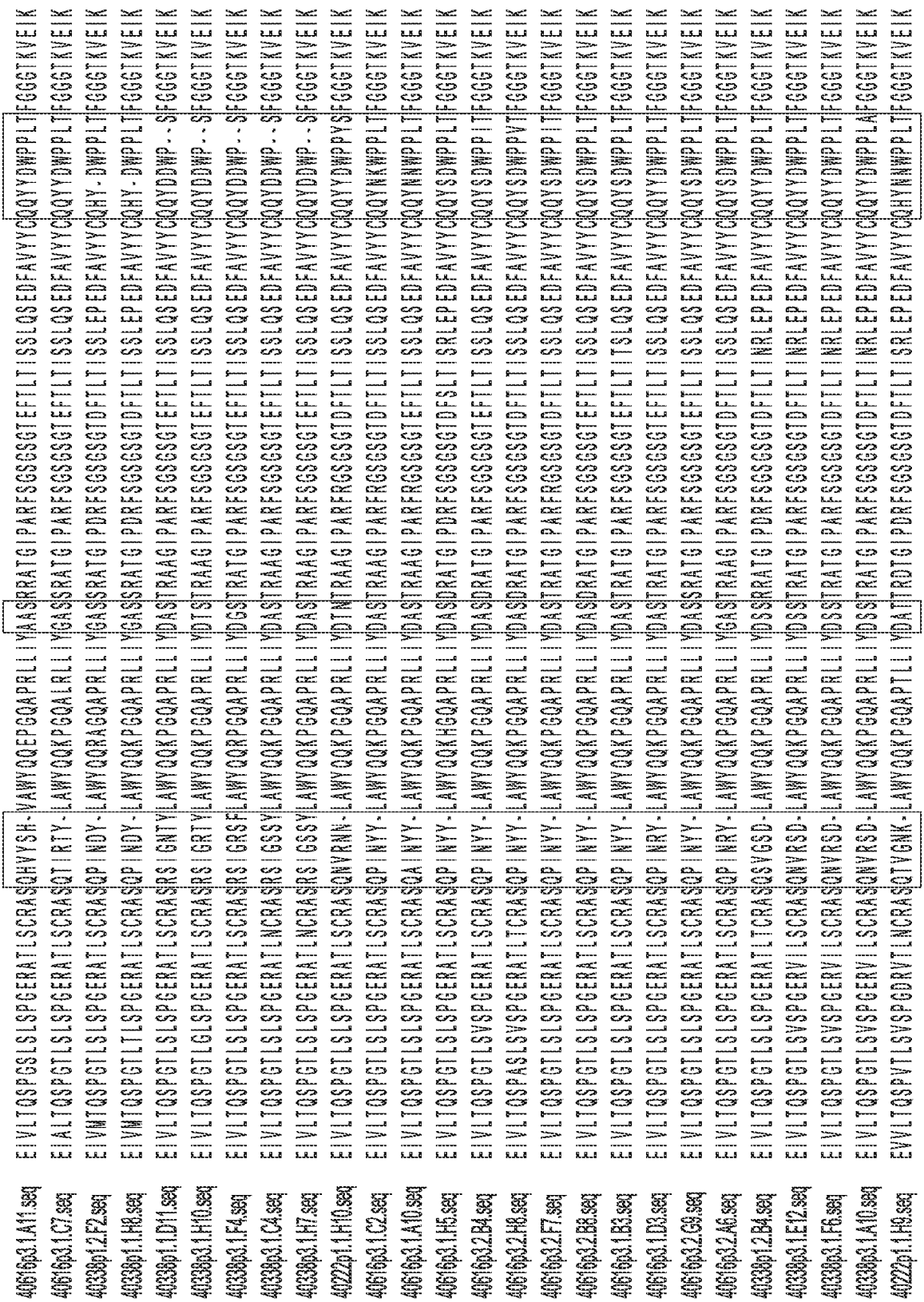

CDR1
CDR2
CDR3
40616p3.1A11.seq EIVLTQSPGSLSLSPGERATLSCRASQHVYSH-VAWYQQKPGQAPRLLIYAASRRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40616p3.1C7.seq EIALTQSPGTLSLSPGERATLSCRASQTIRTY-LAWYQQKPGQALRLLIYGASSRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40338p1.2F2.seq EIVMTQSPGTLSLSPGERATLSCRASQPINDY-LAWYQQRAGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISSLEPEDFAVYYCQHY-DWPPLTFGGGTKVEIK
40338p1.1H8.seq EIVMTQSPGTLTLSPGERATLSCRASQPINDY-LAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISSLEPEDFAVYYCQHY-DWPPLTFGGGTKVEIK
40338p1.1D11.seq EIVLTQSPGTLSLSPGERATLSCRASRSIGNTYLAWYQQKPGQAPRLLIYDASTRAAGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYDDWP--SFGGGTKVEIK
40338p3.1H10.seq EIVLTQSPGTLGLSPGERATLSCRASRSIGRTYLAWYQQKPGQAPRLLIYDTSTRAAGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYDDWP--SFGGGTKVEIK
40338p3.1F4.seq EIVLTQSPGTLSLSPGERATLSCRASRSIGRSFLAWYQQKPGQAPRLLIYDGSTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYDDWP--SFGGGTKVEIK
40338p3.1C4.seq EIVLTQSPGTLSLSPGERATLNCRASRSIGSSYLAWYQQKPGQAPRLLIYDASTRAAGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYDDWP--SFGGGTKVEIK
40338p3.1H7.seq EIVLTQSPGTLSLSPGERATLNCRASRSIGSSYLAWYQQKPGQAPRLLIYDASTRAAGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYDDWP--SFGGGTKVEIK
40222p1.1H10.seq EIVLTQSPGTLSLSPGERATLSCRASQNVRNN-LAWYQQKPGQAPRLLIYDTNTRAAGIPARFRGSGSGTDFTLTISSLQSEDFAVYYCQQYYDWPPYSFGGGTKVEIK
40616p3.1C2.seq EIVLTQSPGTLSLSPGERATLSCRASQPINYY-LAWYQQKPGQAPRLLIYDASTRAAGIPARFRGSGSGTDFTLTISSLQSEDFAVYYCQQYWKWPPLTFGGGTKVEIK
40616p3.1A10.seq EIVLTQSPGTLSLSPGERATLSCRASQAINYY-LAWYQQKPGQAPRLLIYDASTRAAGIPARFRGSGSGTEFTLTISSLQSEDFAVYYCQQYWNWPPLTFGGGTKVEIK
40616p3.1H5.seq EIVLTQSPGTLSLSPGERATLSCRASQPINYY-LAWYQQKHGQAPRLLIYDASDRATGIPDRFSGSGSGTDFSLTISRLEPEDFAVYYCQQYSDWPPLTFGGGTKVEIK
40616p3.2B4.seq EIVLTQSPGTLSVSPGERATLSCRASQPINYY-LAWYQQKPGQAPRLLIYDASDRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYSDWPPITFGGGTKVEIK
40616p3.2H8.seq EIVLTQSPASLSVSPGERATLTCRASQPINYY-LAWYQQKPGQAPRLLIYDASDRATGIPARFSGSGSGTDFTLTISSLQSEDFAVYYCQQYSDWPPVTFGGGTKVEIK
40616p3.2F7.seq EIVLTQSPGTLSLSPGERATLSCRASQPINYY-LAWYQQKPGQAPRLLIYDASTRATGIPARFRGSGSGTDFTLTISSLQSEDFAVYYCQQYSDWPPITFGGGTKVEIK
40616p3.2B8.seq EIVLTQSPGTLSLSPGERATLSCRASQPINYY-LAWYQQKPGQAPRLLIYDASDRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYSDWPPLTFGGGTKVEIK
40616p3.1B3.seq EIVLTQSPGTLSLSPGERATLSCRASQPINYY-LAWYQQKPGQAPRLLIYDASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYSDWPPLTFGGGTKVEIK
40616p3.1D3.seq EIVLTQSPGTLSLSPGERATLSCRASQPINRY-LAWYQQKPGQAPRLLIYDASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYSDWPPLTFGGGTKVEIK
40616p3.2G9.seq EIVLTQSPGTLSLSPGERATLSCRASQPINYY-LAWYQQKPGQAPRLLIYDASSRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYSDWPPLTFGGGTKVEIK
40616p3.2A6.seq EIVLTQSPGTLSLSPGERATLSCRASQPINRY-LAWYQQKPGQAPRLLIYGASTRAAGIPARFSGSGSGTDFTLTISSLQSEDFAVYYCQQYSDWPPLTFGGGTKVEIK
40338p1.2B4.seq EIVLTQSPGTLSLSPGERATLTCRASQSVYGSD-LAWYQQKPGQAPRLLIYDSSRRATGIPDRFSGSGSGTDFTLTINRLEPEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40338p3.1E12.seq EIVLTQSPGTLSVSPGERVILSCRASQNVRSD-LAWYQQKPGQAPRLLIYDSSTRATGIPARFSGSGSGTDFTLTINRLEPEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40338p3.1F6.seq EIVLTQSPGTLSVSPGERVILSCRASQNVRSD-LAWYQQKPGQAPRLLIYDSSTRATGIPARFSGSGSGTDFTLTINRLEPEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40338p3.1A10.seq EIVLTQSPGTLSVSPGERVILSCRASQNVRSD-LAWYQQKPGQAPRLLIYDSSTRATGIPARFSGSGSGTDFTLTINRLEPEDFAVYYCQQYYDWPPLAFGGGTKVEIK
40222p1.1H9.seq EVVLTQSPVTLSVSPGDRVTLNCRASQTVGNK-LAWYQQKPGQAPTLLIYDATTRDTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYWNWPPLTFGGGTKVEIK

FIG. 1 (Cont.)

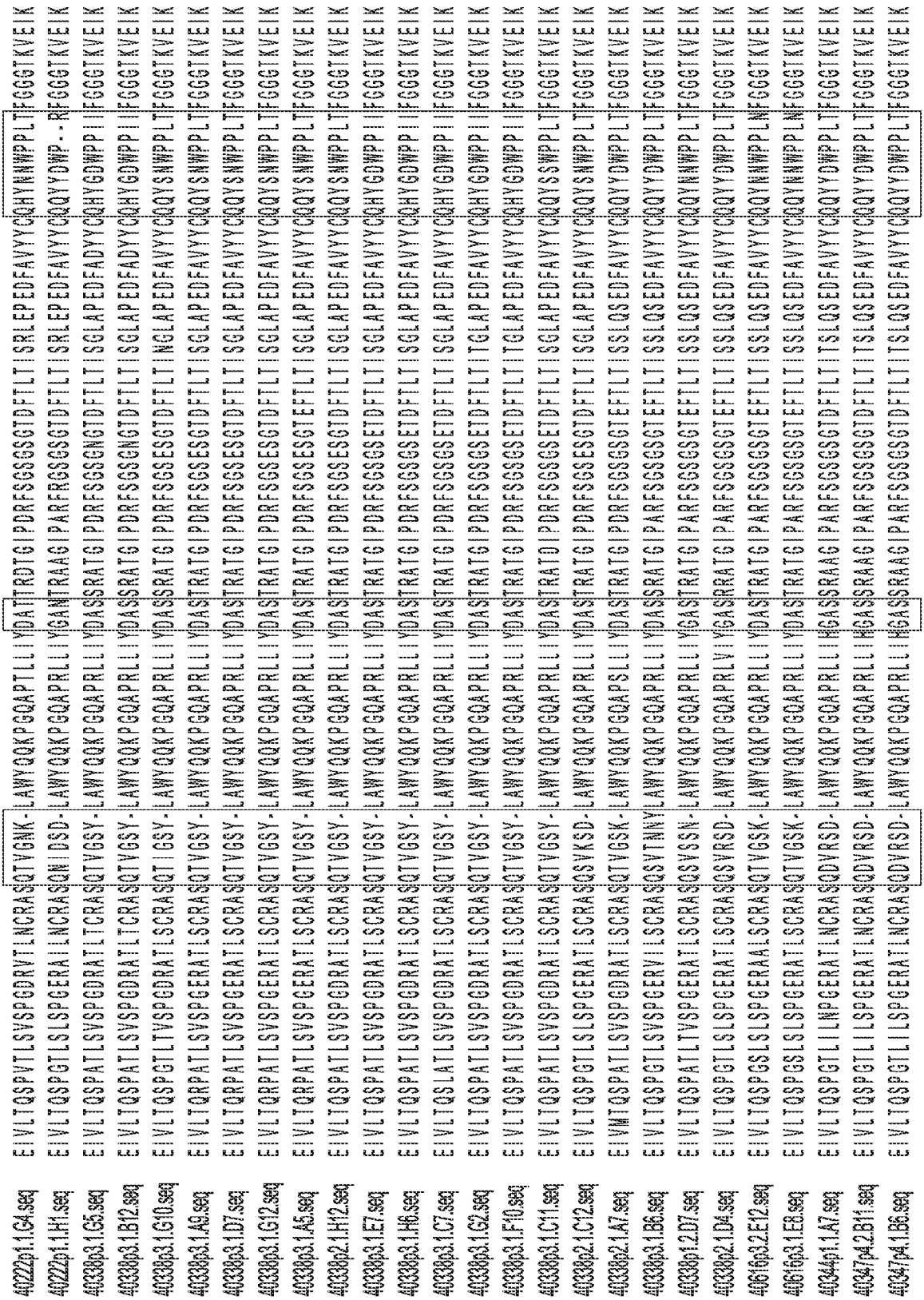

CDR1
CDR2
CDR3
40222p1.1.G4.seq EIVLTQSPVTLSVSPGDRVTLNCRASQTVGNK-LAWYQQKPGQAPTLLIYDATTRDTGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQHYNNWPPLTFGGGTKVEIK
40222p1.1.H1.seq EIVLTQSPGTLSLSPGERATLNCRASQNIDSD-LAWYQQKPGQAPRLLIYGANTRAAGIPARFRGSGSGTDFTLTISRLEPEDFAVYYCQQYYDWP--RFGGGTKVEIK
40338p3.1.G5.seq EIVLTQSPATLSVSPGDRATLTCRASQTVGSY-LAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSGNGTDFTLTISGLAPEDFADYYCQHYGDWPPIIFGGGTKVEIK
40338p3.1.B12.seq EIVLTQSPATLSVSPGDRATLTCRASQTVGSY-LAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSGNGTDFTLTISGLAPEDFADYYCQHYGDWPPIIFGGGTKVEIK
40338p3.1.G10.seq EIVLTQSPGTLTVSPGDRATLSCRASQTIGSY-LAWYQQKPGQAPRLLIYDASSRATGIPDRFSGSESGTDFTLTINGLAPEDFAVYYCQQYSNWPPLTFGGGTKVEIK
40338p3.1.A9.seq EIVLTQRPATLSVSPGERATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSESGTDFTLTISGLAPEDFAVYYCQQYSNWPPLTFGGGTKVEIK
40338p3.1.D7.seq EIVLTQRPATLSVSPGERATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSESGTDFTLTISGLAPEDFAVYYCQQYSNWPPLTFGGGTKVEIK
40338p3.1.G12.seq EIVLTQRPATLSVSPGERATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSESGTDFTLTISGLAPEDFAVYYCQQYSNWPPLTFGGGTKVEIK
40338p3.1.A5.seq EIVLTQRPATLSVSPGERATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSESGTEFTLTISGLAPEDFAVYYCQQYSNWPPLTFGGGTKVEIK
40338p2.1.H12.seq EIVLTQSPATLSVSPGDRATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSESGTDFTLTISGLAPEDFAVYYCQQYSNWPPLTFGGGTKVEIK
40338p3.1.E7.seq EIVLTQSPATLSVSPGDRATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSETDFTLTISGLAPEDFAVYYCQHYGDWPPIIFGGGTKVEIK
40338p3.1.H6.seq EIVLTQSPATLSVSPGDRATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSETDFTLTISGLAPEDFAVYYCQHYGDWPPIIFGGGTKVEIK
40338p3.1.C7.seq EIVLTQSLATLSVSPGDRATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSETDFTLTISGLAPEDFAVYYCQHYGDWPPIIFGGGTKVEIK
40338p3.1.G2.seq EIVLTQSPATLSVSPGDRATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSETDFTLTITGLAPEDFAVYYCQHYGDWPPIIFGGGTKVEIK
40338p3.1.F10.seq EIVLTQSPATLSVSPGDRATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSGSETDFTLTITGLAPEDFAVYYCQHYGDWPPIIFGGGTKVEIK
40338p3.1.C11.seq EIVLTQSPATLSVSPGDRATLSCRASQTVGSY-LAWYQQKPGQAPRLLIYDASTRATDIPDRFSGSGSETDFTLTISGLAPEDFAVYYCQQYSSWPPLTFGGGTKVEIK
40338p2.1.C12.seq EIVLTQSPGTLSLSPGERATLSCRASQSVKSD-LAWYQQKPGQAPRLLIYDASTRATGIPDRFSGSESGTDFTLTISGLAPEDFAVYYCQQYSNWPPLTFGGGTKVEIK
40338p2.1.A7.seq EIVMTQSPATLSVSPGDRATLSCRASQTVGSK-LAWYQQKPGQAPSLLIYDASTRATGIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40338p3.1.B6.seq EIVLTQSPGTLSVSPGERVTLSCRASQSVTNNY LAWYQQKPGQAPRLLIYDASSRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYTDWPPLTFGGGTKVEIK
40338p1.2.D7.seq EIVLTQSPATLTVSPGERATLSCRASQSVSSN-LAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPLTFGGGTKVEIK
40338p2.1.D4.seq EIVLTQSPGTLSLSPGERATLSCRASQSVYRSD-LAWYQQKPGQAPRLVIYGASSRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYTDWPPLTFGGGTKVEIK
40616p3.2.E12.seq EIVLTQSPGSLSLSPGERAASCRASQTVGSK-LAWYQQKPGQAPRLLIYDASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPLNFGGGTKVEIK
40616p3.1.E8.seq EIVLTQSPGSLSLSPGERATLSCRASQTVGSK-LAWYQQKPGQAPRLLIYDASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPLNFGGGTKVEIK
40344p1.1.A7.seq EIVLTQSPGTLILNPGERATLNCRASQDVRSD-LAWYQQKPGQAPRLLIHGASSRAAGIPARFSGSGSGTDFTLTITSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40347p4.2.B11.seq EIVLTQSPGTLILSPGERATLNCRASQDVRSD-LAWYQQKPGQAPRLLIHGASSRAAGIPARFSGSGSGTDFTLTITSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40347p4.1.B6.seq EIVLTQSPGTLILSPGERATLNCRASQDVRSD-LAWYQQKPGQAPRLLIHGASSRAAGIPARFSGSGSGTDFTLTITSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK

FIG. 1 (Cont.)

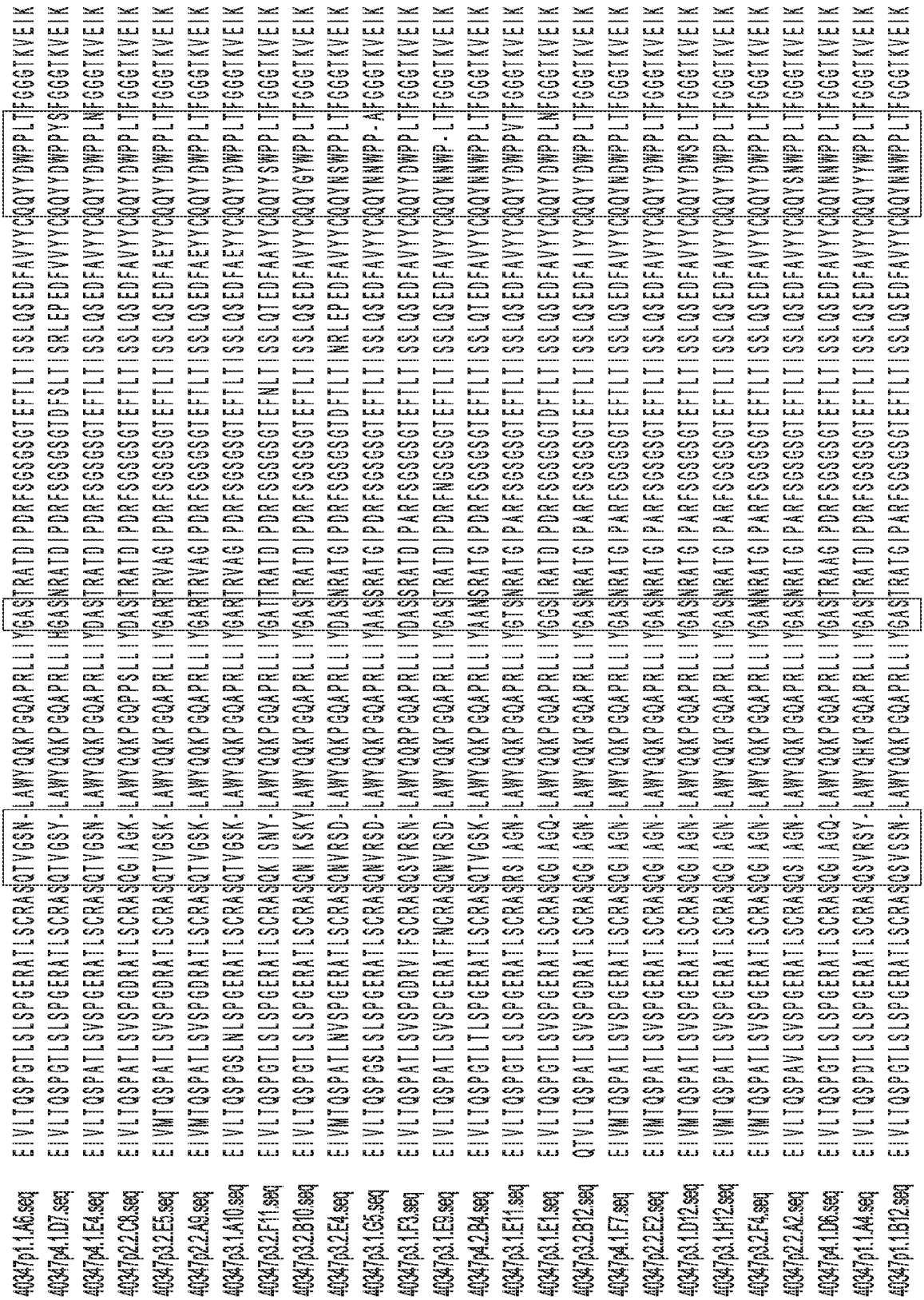

CDR1
CDR2
CDR3
4D347p1.1A6.seq EIVLTQSPGTLSLSPGERATLSCRASQTVGSN-LAWYQQKPGQAPRLLIYGASTRATDIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
4D347p4.1D7.seq EIVLTQSPGTLSLSPGERATLSCRASQTVGSY-LAWYQQKPGQAPRLLIHGASNRATDIPDRFSGSGSGTDFSLTISRLEPEDFVVYYCQQYYDWPPYSFGGGTKVEIK
4D347p4.1E4.seq EIVLTQSPATLSVSPGERATLSCRASQTVGSN-LAWYQQKPGQAPRLLIYDASTRATDIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLNFGGGTKVEIK
4D347p2.2C8.seq EIVLTQSPATLSVSPGDRATLSCRASQGIAGK-LAWYQQKPGQPPSLLIYDASTRATDIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
4D347p3.2E5.seq EIVMTQSPATLSVSPGDRATLSCRASQTVGSK-LAWYQQKPGQAPRLLIYGARTRVAGIPDRFSGSGSGTEFTLTISSLQSEDFAEYYCQQYYDWPPLTFGGGTKVEIK
4D347p2.2A9.seq EIVMTQSPATLSVSPGDRATLSCRASQTVGSK-LAWYQQKPGQAPRLLIYGARTRVAGIPDRFSGSGSGTEFTLTISSLQSEDFAEYYCQQYYDWPPLTFGGGTKVEIK
4D347p3.1A10.seq EIVLTQSPGSLNLSPGERATLSCRASQTVGSK-LAWYQQKPGQAPRLLIYGARTRVAGIPDRFSGSGSGTEFTLTISSLQSEDFAEYYCQQYYDWPPLTFGGGTKVEIK
4D347p3.2F11.seq EIVLTQSPGTLSLSPGERATLSCRASQKISNY-LAWYQQKPGQAPRLLIYGATTRATDIPDRFSGSGSGTEFNLTISSLQTEDFAAYYCQQYYSWPPLTFGGGTKVEIK
4D347p3.2B10.seq EIVLTQSPGTLSLSPGERATLSCRASQNIKSKYLAWYQQKPGQAPRLLIYGASTRATDIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQGYGYWPPLTFGGGTKVEIK
4D347p3.2E4.seq EIVMTQSPATLNVSPGERATLSCRASQNVRSD-LAWYQQKPGQAPRLLIYDASNRATGIPDRFSGSGSGTDFTLTINRLEPEDFAVYYCQQYNSWPPLTFGGGTKVEIK
4D347p3.1G5.seq EIVLTQSPGSLSLSPGERATLSCRASQNVRSD-LAWYQQKPGQAPRLLIYAASSRATGIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPP-AFGGGTKVEIK
4D347p3.1F3.seq EIVLTQSPATLSVSPGDRVIFSCRASQSVRSN-LAWYQQRPGQAPRLLIYDASSRATDIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
4D347p3.1E9.seq EIVLTQSPATLSVSPGERATFNCRASQNVRSD-LAWYQQKPGQAPRLLIYGASTRATDIPDRFNGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPP-LTFGGGTKVEIK
4D347p4.2B4.seq EIVLTQSPGTLTLSPGERATLSCRASQTVGSK-LAWYQQKPGQAPRLLIYAANSRATGIPDRFSGSGSGTEFTLTISSLQTEDFAVYYCQQYNNWPPLTFGGGTKVEIK
4D347p3.1E11.seq EIVLTQSPGTLSLSPGERATLSCRASRSIAGN-LAWYQQKPGQAPRLLIYGTSNRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPVTFGGGTKVEIK
4D347p3.1E1.seq EIVLTQSPGTLSVSPGERATLSCRASQSIAGQ-LAWYQQKPGQAPRLLIYGGSSRATDIPDRFSGSGSGTDFTLTISSLQSEDFAVYYCQQYYDWPPLNFGGGTKVEIK
QIVLTQSPATLSVSPGDRATLSCRASQSIAGN-LAWYQQKPGQAPRLLIYGASNRATGIPARFSGSGSGTEFTLTISSLQSEDFAIYYCQQYYDWPPLTFGGGTKVEIK
4D347p3.2B12.seq EIVMTQSPATLSVSPGERATLSCRASQSIAGN-LAWYQQKPGQAPRLLIYGASNRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNDWPPLTFGGGTKVEIK
4D347p4.1F7.seq EIVMTQSPATLSVSPGERATLSCRASQGIAGN-LAWYQQKPGQAPRLLIYGASNRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
4D347p2.2E2.seq EIVMTQSPATLSVSPGERATLSCRASQGIAGN-LAWYQQKPGQAPRLLIYGASNRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
4D347p3.1D12.seq EIVMTQSPATLSVSPGERATLSCRASQGIAGN-LAWYQQKPGQAPRLLIYGASNRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWSPLTFGGGTKVEIK
4D347p3.1H12.seq EIVMTQSPATLSVSPGERATLSCRASQGIAGN-LAWYQQKPGQAPRLLIYGASNRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
4D347p3.2F4.seq EIVMTQSPATLSVSPGERATLSCRASQGIAGN-LAWYQQKPGQAPRLLIYGANNRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
4D347p2.2A2.seq EIVLTQSPAVLSVSPGERATLSCRASQSIAGN-LAWYQQKPGQAPRLLIYGASNRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYSNWPPLTFGGGTKVEIK
4D347p4.1D6.seq EIVLTQSPGTLSLSPGERATLSCRASQGIAGQ-LAWYQQKPGQAPRLLIYGASTRAAGIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPLTFGGGTKVEIK
4D347p1.1A4.seq EIVLTQSPDTLSLSPGERATLSCRASQSVRSY-LAWYQHKPGQAPRLLIYGASTRATDIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYYWPPLTFGGGTKVEIK
4D347p1.1B12.seq EIVLTQSPGTLSLSPGERATLSCRASQSVSSN-LAWYQQKPGQAPRLLIYGASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPLTFGGGTKVEIK

FIG. 1 (Cont.)

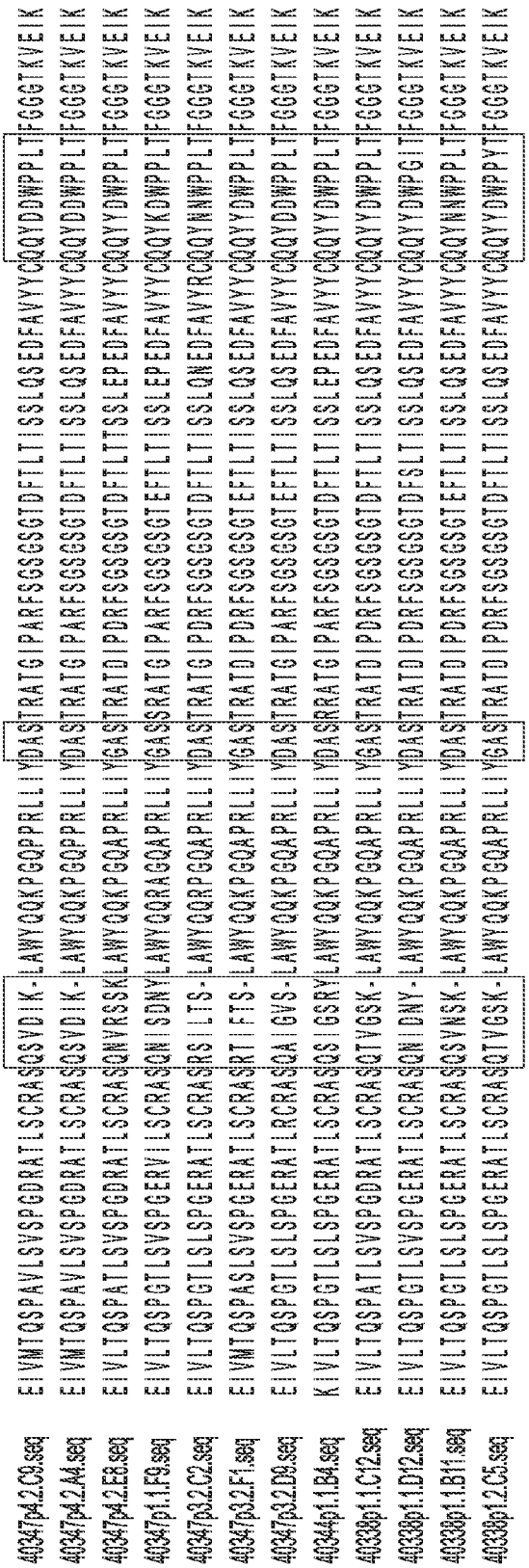

CDR1
CDR2
CDR3
40347p4.2.C9.seq EIVMTQSPAVLSVSPGDRATLSCRASQSVDIK-LAWYQQKPGQPPRLLIYDASTRATGIPARFSGSGSGTDFTLTISSLQSEDFAVYYCQQYDDWPPLTFGGGTKVEIK
40347p4.2.A4.seq EIVMTQSPAVLSVSPGDRATLSCRASQSVDIK-LAWYQQKPGQPPRLLIYDASTRATGIPARFSGSGSGTDFTLTISSLQSEDFAVYYCQQYDDWPPLTFGGGTKVEIK
40347p4.2.E8.seq EIVLTQSPATLSVSPGDRATLSCRASQNVRSSK-LAWYQQKPGQAPRLLIYGASTRATDIPDRFSGSGSGTDFTLTSSLEPEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40347p1.1.F9.seq EIVLTQSPGTLSVSPGERVILSCRASQNISDNY-LAWYQQRAGQAPRLLIYGASSRATGIPARFSGSGSGTEFTLTISSLEPEDFAVYYCQQYKDWPPLTFGGGTKVEIK
40347p3.2.C2.seq EIVLTQSPGTLSLSPGERATLSCRASRSILTS-LAWYQQRPGQAPRLLIYDASTRATGIPDRFSGSGSGTDFTLTISSLQNEDFAVYRCQQYNNWPPLTFGGGTKVEIK
40347p3.2.F1.seq EIVMTQSPASLSVSPGERATLSCRASRTIFTS-LAWYQQKPGQAPRLLIYGASTRATDIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40347p3.2.D9.seq EIVLTQSPGTLSLSPGERATLRCRASQAIGVS-LAWYQQKPGQAPRLLIYDASTRATGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCQQYDDWPPLTFGGGTKVEIK
40344p1.1.B4.seq KIVLTQSPGTLSLSPGERATLSCRASQSIGSRY-LAWYQQKPGQAPRLLIYDASRRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40338p1.1.C12.seq EIVLTQSPATLSVSPGDRATLSCRASQTVGSK-LAWYQQKPGQAPRLLIYGASTRATDIPDRFSGSGSGTDFTLTISSLQSEDFAVYYCQQYYDWPPLTFGGGTKVEIK
40338p1.1.D12.seq EIVLTQSPGTLSVSPGERATLSCRASQNIDNY-LAWYQQKPGQAPRLLIYDASTRATDIPDRFSGSGSGTDFSLTISSLQSEDFAVYYCQQYYDWPGITFGGGTKVEIK
40338p1.1.B11.seq EIVLTQSPGTLSLSPGERATLSCRASQSVNSK-LAWYQQKPGQAPRLLIYDASTRATDIPDRFSGSGSGTEFTLTISSLQSEDFAVYYCQQYNNWPPLTFGGGTKVEIK
40338p1.2.C5.seq EIVLTQSPGTLSLSPGERATLSCRASQTVGSK-LAWYQQKPGQAPRLLIYGASTRATDIPDRFSGSGSGTDFTLTISSLQSEDFAVYYCQQYYDWPPYTFGGGTKVEIK

FIG. 2

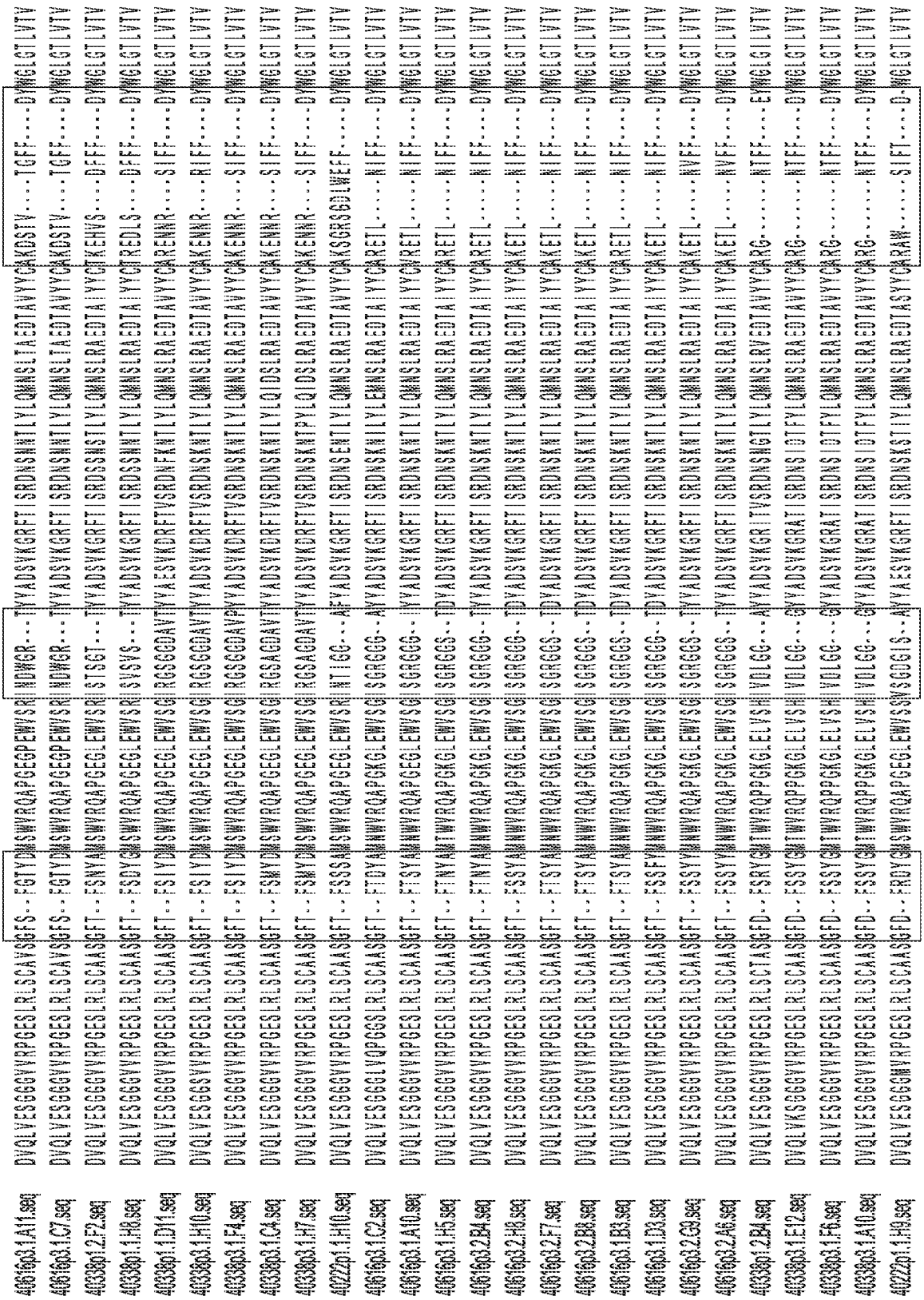

CDR1
CDR2
CDR3
40616p3.1A11.seq
40616p3.1C7.seq
40336p1.2F2.seq
40336p1.1H8.seq
40336p1.1D11.seq
40336p3.1H10.seq
40336p3.1F4.seq
40336p3.1C4.seq
40336p3.1H7.seq
40222p1.1H10.seq
40616p3.1C2.seq
40616p3.1A10.seq
40616p3.1H5.seq
40616p3.2B4.seq
40616p3.2H8.seq
40616p3.2F7.seq
40616p3.2B8.seq
40616p3.1B3.seq
40616p3.1D3.seq
40616p3.2G9.seq
40616p3.2A6.seq
40336p1.2B4.seq
40336p3.1E12.seq
40336p3.1F6.seq
40336p3.1A10.seq
40222p1.1H9.seq

FIG. 2 (Cont.)

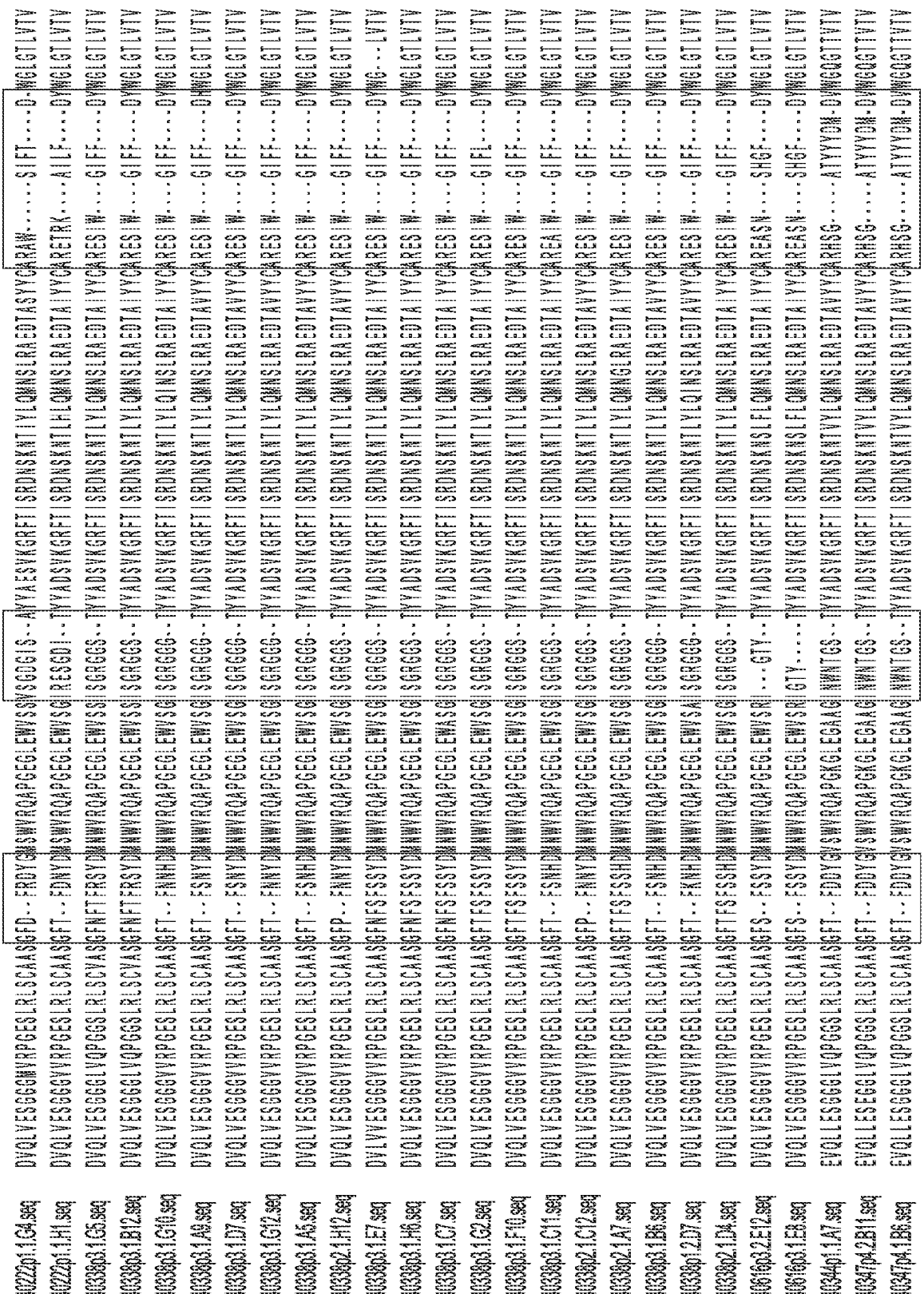
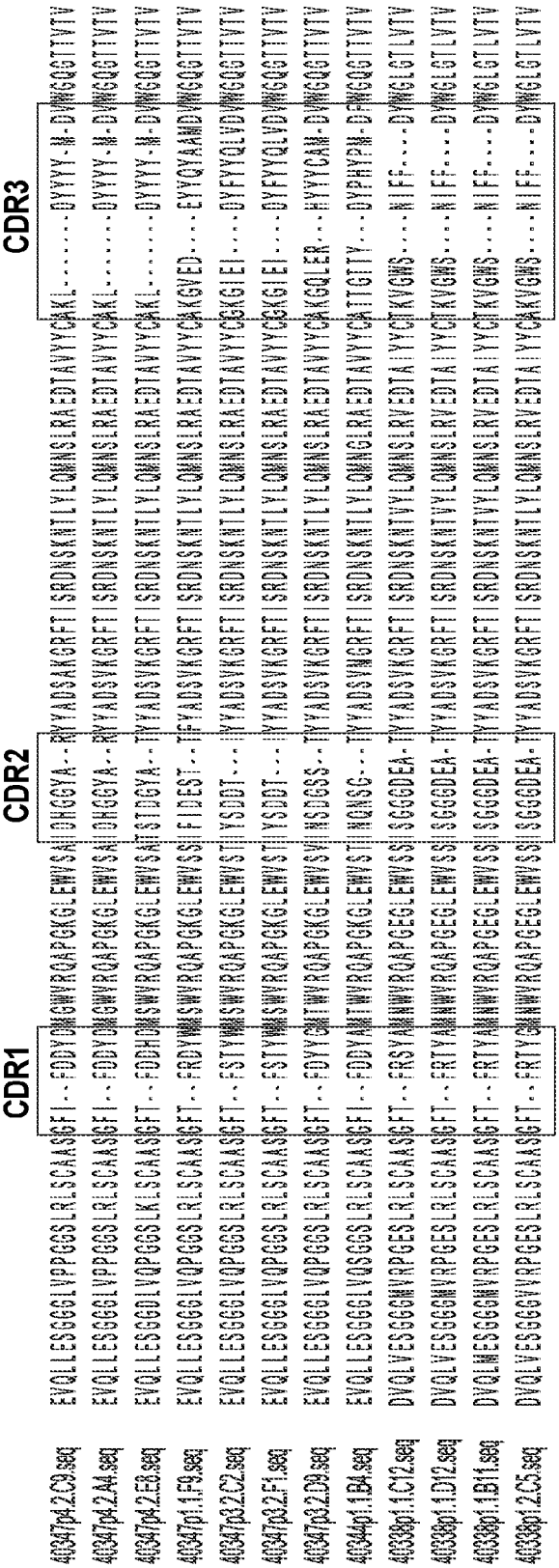


FIG. 2 (Cont.)

FIG. 2 (Cont.)


CDR1
CDR2
CDR3
40347p4.2.C3.seq EVQLLESGGGLVQPGGSLRLSCAASGFI--FDYCMGWVRQAPGKGLEWVSAI-DHGGYA--RYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKL------DYYYY-N-DVWGQGTTVTV
40347p4.2.A4.seq EVQLLESGGGLVQPGGSLRLSCAASGFI--FDYCMGWVRQAPGKGLEWVSAI-DHGGYA--RYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKL------DYYYY-N-DVWGQGTTVTV
40347p4.2.E8.seq EVQLLESGGGLVQPGGSLKLSCAASGFT--FDDHCMSWVRQAPGKGLEWVSAI-STDGYA--TYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKL------DYYYY-N-DVWGQGTTVTV
40347p1.1.F9.seq EVQLLESGGGLVQPGGSLRLSCAASGFT--FRDYWMSWVRQAPGKGLEWVSSI-FIDSST--TFYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKGVED----EYVQYAAMDVWGQGTTVTV
40347p3.2.C2.seq EVQLLESGGGLVQPGGSLRLSCAASGFT--FSTYWMSWVRQAPGKGLEWVST-IYSDDT----IYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCDKGIEL----DYFVYQLVDVWGQGTTVTV
40347p3.2.F1.seq EVQLLESGGGLVQPGGSLRLSCAASGFT--FSTYWMSWVRQAPGKGLEWVST-IYSDDT----IYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCDKGIEL----DYFVYQLVDVWGQGTTVTV
40347p3.2.D9.seq EVQLLESGGGLVQPGGSLRLSCAASGFT--FDYYCMTWVRQAPGKGLEWVSYI-NSDGSS--TYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARKGQLER---HYYYDAM-DVWGQGTTVTV
40344p1.1.B4.seq EVQLLESGGGLVQSGGSLRLSCAASGFI--FDDYAMTWVRQAPGKGLEWVST-INQNSG----TYYADSVWGRFTISRDNSKNTLYLQMNGLRAEDTAVYYCAPTTGTTY---DYPHYPM-DFWGQGTTVTV
40338p1.1.C12.seq DVQLVESGGGWVRPGESLRLSCAASGFT--FRSYAMNWVRQAPGEGLEWVSSI-SGGGDEA-TPYYADSVKGRFTISRDNSKNTVYLQMNSLRVEDTAIYYCTKVGWS----NIFF----DYWGLGTLVTV
40338p1.1.D12.seq DVQLVESGGGWVRPGESLRLSCAASGFT--FRTYAMNWVRQAPGEGLEWVSSI-SGGGDEA-TPYYADSVKGRFTISRDNSKNTVYLQMNSLRVEDTAIYYCTKVGWS----NIFF----DYWGLGTLVTV
40338p1.1.B11.seq DVQLMESGGGWVRPGESLRLSCAASGFT--FRTYAMNWVRQAPGEGLEWVSSI-SGGGDEA-TPYYADSVKGRFTISRDNSKNTVYLQMNSLRVEDTAIYYCTKVGWS----NIFF----DYWGLGTLVTV
40338p1.2.C5.seq DVQLVESGGGVVRPGESLRLSCAASGFT--FRTYGMNWVRQAPGEGLEWVSSI-SGGGDEA-TPYYADSVKGRFTISRDNSKNTLYLQMNSLRVEDTAIYYCTKVGWS----NIFF----DYWGLGTLVTV

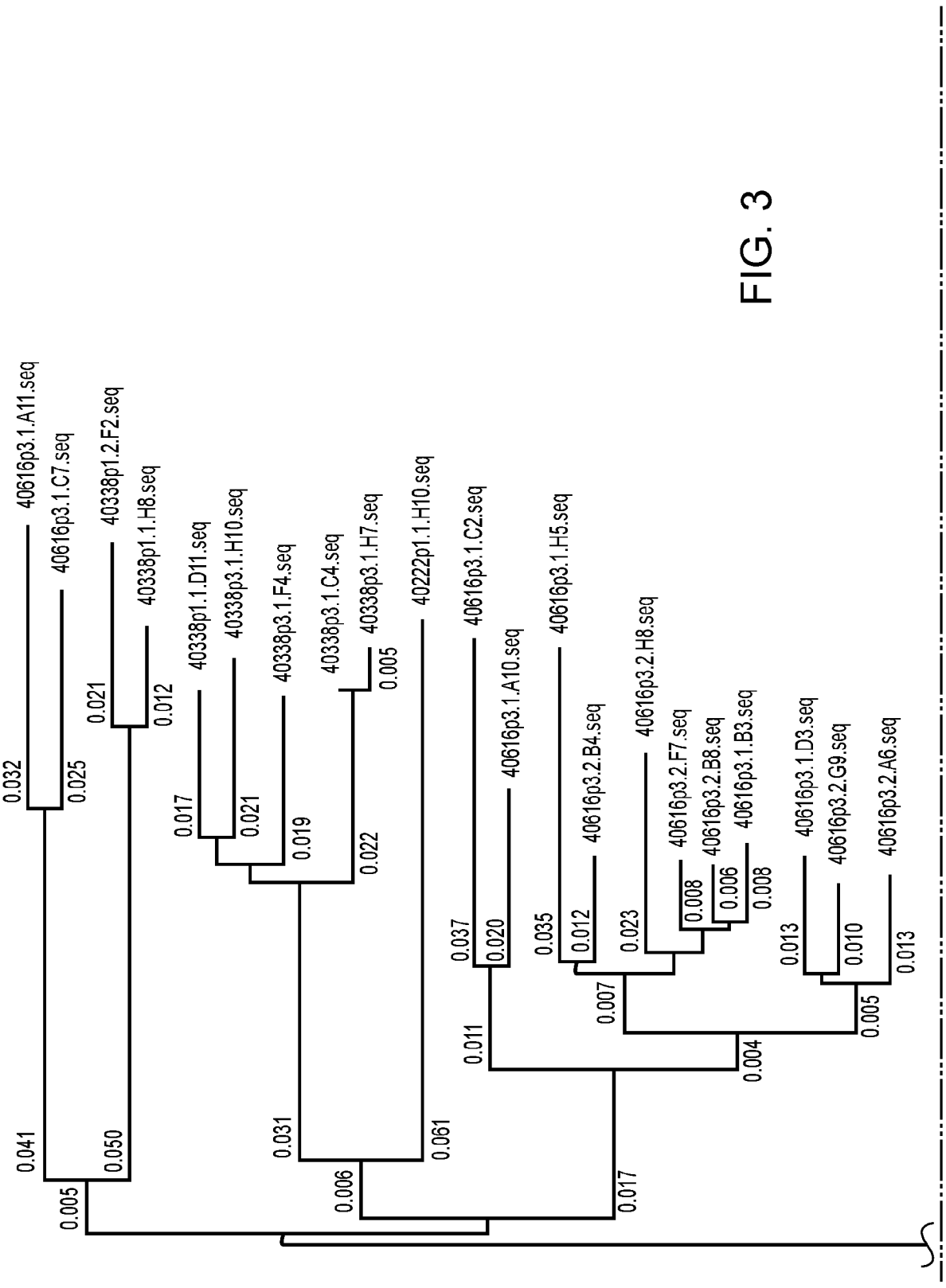

40616p3.1.A11.seq
40616p3.1.C7.seq
40338p1.2.F2.seq
40338p1.1.H8.seq
40338p1.1.D11.seq
40338p3.1.H10.seq
40338p3.1.F4.seq
40338p3.1.C4.seq
40338p3.1.H7.seq
40222p1.1.H10.seq
40616p3.1.C2.seq
40616p3.1.A10.seq
40616p3.1.H5.seq
40616p3.2.B4.seq
40616p3.2.H8.seq
40616p3.2.F7.seq
40616p3.2.B8.seq
40616p3.1.B3.seq
40616p3.1.D3.seq
40616p3.2.G9.seq
40616p3.2.A6.seq
0.032
0.025
0.021
0.012
0.017
0.021
0.019
0.005
0.022
0.041
0.050
0.031
0.061
0.006
0.037
0.020
0.035
0.012
0.023
0.008
0.006
0.008
0.011
0.004
0.013
0.010
0.007
0.005
0.013
0.017

FIG. 3

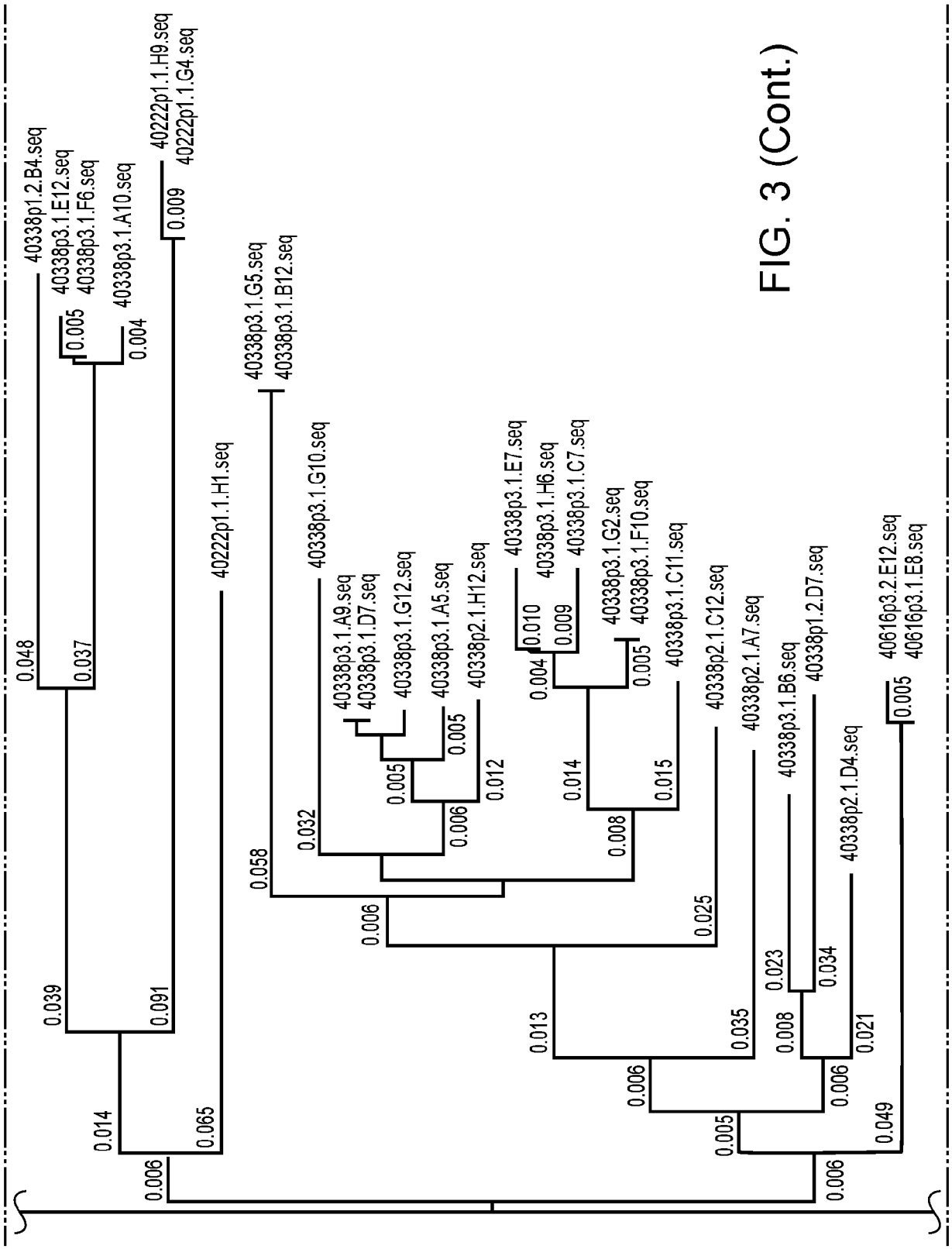

40338p1.2.B4.seq
40338p3.1.E12.seq
40338p3.1.F6.seq
40338p3.1.A10.seq
40222p1.1.H9.seq
40222p1.1.G4.seq
40222p1.1.H1.seq
40338p3.1.G5.seq
40338p3.1.B12.seq
40338p3.1.G10.seq
40338p3.1.A9.seq
40338p3.1.D7.seq
40338p3.1.G12.seq
40338p3.1.A5.seq
40338p2.1.H12.seq
40338p3.1.E7.seq
40338p3.1.H6.seq
40338p3.1.C7.seq
40338p3.1.G2.seq
40338p3.1.F10.seq
40338p3.1.C11.seq
40338p2.1.C12.seq
40338p2.1.A7.seq
40338p3.1.B6.seq
40338p1.2.D7.seq
40338p2.1.D4.seq
40616p3.2.E12.seq
40616p3.1.E8.seq
0.048
0.005
0.004
0.009
0.037
0.039
0.091
0.014
0.065
0.006
0.058
0.032
0.005
0.005
0.006
0.012
0.004
0.010
0.009
0.014
0.008
0.005
0.015
0.025
0.006
0.013
0.035
0.023
0.008
0.034
0.006
0.021
0.005
0.049
0.006

FIG. 3 (Cont.)

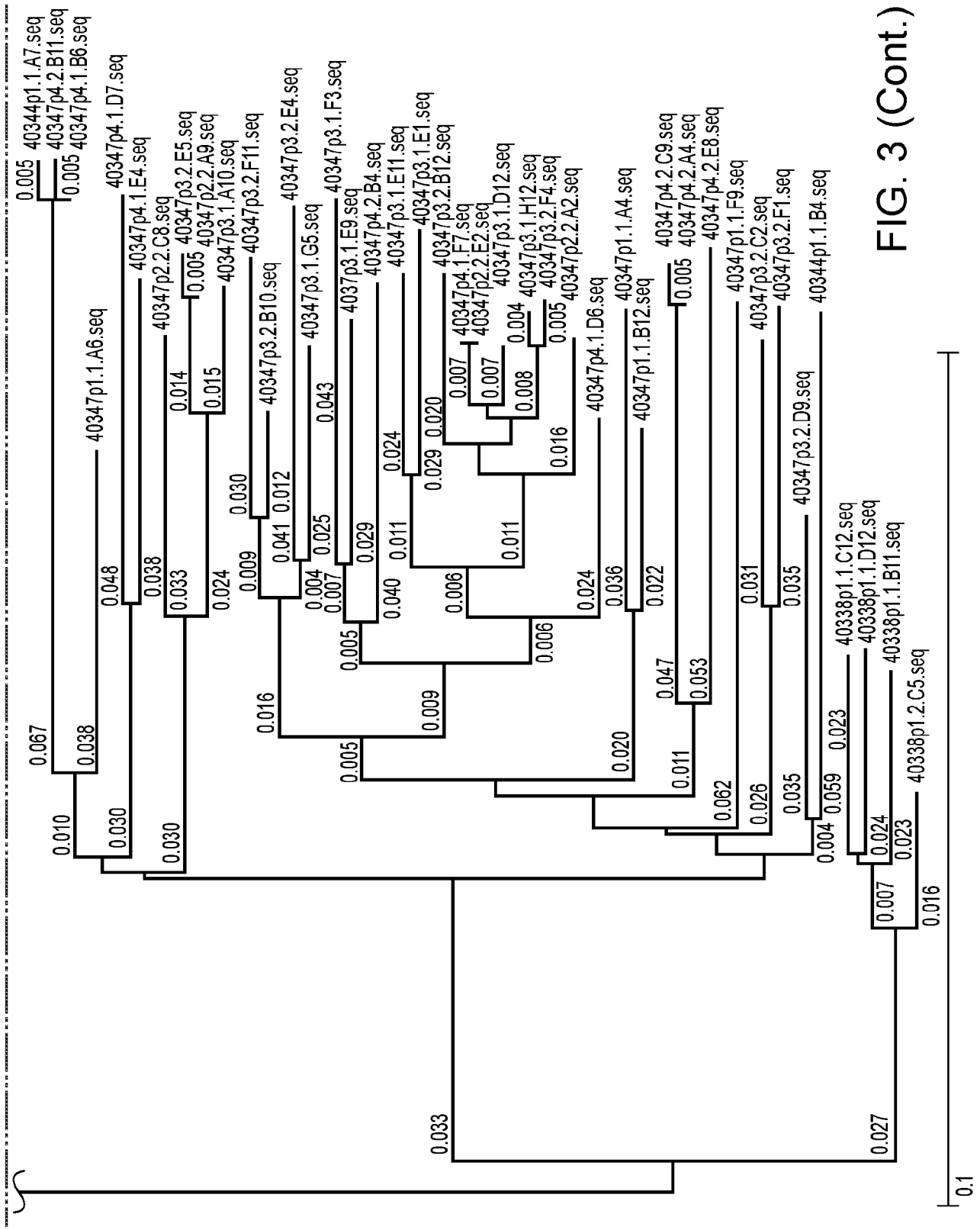

40344p1.1.A7.seq
40347p4.2.B11.seq
40347p4.1.B6.seq
40347p4.1.D7.seq
40347p4.1.E4.seq
40347p2.2.C8.seq
40347p3.2.E5.seq
40347p2.2.A9.seq
40347p3.1.A10.seq
40347p3.2.F11.seq
40347p1.1.A6.seq
40347p3.2.B10.seq
40347p3.2.E4.seq
40347p3.1.G5.seq
40347p3.1.F3.seq
40347p3.1.E9.seq
40347p4.2.B4.seq
40347p3.1.E11.seq
40347p3.1.E1.seq
40347p3.2.B12.seq
40347p4.1.F7.seq
40347p2.2.E2.seq
40347p3.1.D12.seq
40347p3.1.H12.seq
40347p3.2.F4.seq
40347p2.2.A2.seq
40347p4.1.D6.seq
40347p1.1.A4.seq
40347p1.1.B12.seq
40347p4.2.C9.seq
40347p4.2.A4.seq
40347p4.2.E8.seq
40347p1.1.F9.seq
40347p3.2.C2.seq
40347p3.2.F1.seq
40347p3.2.D9.seq
40344p1.1.B4.seq
40338p1.1.C12.seq
40338p1.1.D12.seq
40338p1.1.B11.seq
40338p1.2.C5.seq
0.1

FIG. 3 (Cont.)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/33226

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61P 25/28, A61P 25/02, A61P 37/02 (2021.01)

CPC - C07K 16/2803, G01N 2333/48, G01N 33/6854, A61K 2039/505, C07K 2317/56

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2014/0179677 A1 (KAWAMURA) 26 June 2014 (26.06.2014) abstract, para [0113]	1-3
A	US 2018/0105600 A1 (ALEXO THERAPEUTICS INC.) 19 April 2018 (19.04.2018) abstract, para [0009], [0048], [0050], SEQ ID NOs: 7	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 October 2021	NOV 04 2021
Name and mailing address of the ISA/US: Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Kari Rodriquez Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/33226

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-17
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

---------- see extra sheet ----------

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, limited to SEQ ID NOs: 1 and 72

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 21/33226

Continuation of Box No. III, Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-3 directed to compositions comprising antibodies that bind to human brain-derived neurotrophic factor (BDNF) and inhibit BDNF signaling, comprising variable domain heavy (VH) and light (VL) chain CDRs (HCDR1-3 and LCDR1-3) and heavy and light chain sequences. The anti-BDNF antibodies will be searched to the extent that the antibody (4Q616p3.1.A11) comprises a VH comprising SEQ ID NO: 1 and a VL comprising SEQ ID NO: 71 (VH and VL CDRs are shown by the applicant in Figs 1 and 2, respectively but are not given SEQ ID NOs). It is believed that claims 1-3 limited to said antibody encompass this first named invention, and thus these claims will be searched without fee to the extent that antibody comprises VH and VL SEQ ID NOs:1 and 71, respectively. Additional anti-BDNF antibodies will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected anti-BDNF antibodies. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an anti-BDNF antibody (40616p3,1.C7) comprising a VH comprising SEQ ID NO: 2 and a VL comprising SEQ ID NO: 72 (claims 1-3).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

No technical features are shared between the anti BDNF antibodies of Group I+, accordingly, these groups lack unity a priori.

Additionally, even if the inventions listed as Group I+ were considered to share technical features, these shared technical features are previously disclosed by the prior art, as further discussed below.

Common Technical Features

The inventions of Group I+ share the technical feature of monoclonal antibodies that bind to human brain-derived neurotrophic factor (BDNF) and inhibit BDNF signaling, comprising heavy and light chain CDRs and heavy and light chain sequences. However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is taught by US 2014/0179677 A1 to Kawamura.

Kawamura teaches monoclonal antibodies (universally known to conprise heavy and light chains, each comprising 3 CDRs) that bind to human brain-derived neurotrophic factor (BDNF) and inhibit BDNF signaling (abstract "The therapeutic agent for ectopic pregnancy contains as an effective ingredient a suppressor of brain-derived neurotrophic factor (BDNF)", para [0113] "As a substance which suppresses the binding between BDNF and TrkB, an antibody to BDNF ... may also be used ... antibodies ... can be obtained by a conventional method comprising administering BDNF ... as an immunogen to an animal (excluding human) to induce an antibody. The antibody may be ... a monoclonal antibody, and the monoclonal antibody can also be prepared by the conventional hybridoma method.").

As the technical feature was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the inventions.

Group I+ therefore lack unity under PCT Rule 13 because they do not share the same or corresponding special technical feature.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 21/33226

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments: