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(54) Title: PREVENTING, TREATING, AND REDUCING (PERSISTENT) POST-TRAUMATIC HEADACHE

Figure 1

Fab	K_D (nM) 1-37 (WT)	K_D (nM) 19-37	K_D (nM) 25-37	K_D (mutant/parent) F27A	V28A	P29A	T30A	N31A	V32A	G33A	S34A	K35A	F37A
7E9	1.0	1.1±0.8	*0.14±0.05*	1.0	1.0	26	7	9	41	125	69	4	3998
8B6	1.1	1.5±1.2	*0.45±0.08*	1.0	1.0	9	2.2	3	5	496	16	3	2527
10A8	2.1	2.4±1.4	*1.0±0.2*	1.0	1.0	9	4	4	11	36	82	13	2152
7D11	4.4	10±7	*3.4±0.4*	1.1	1.0	7	4	5	5	86	18	1.4	420
6H2	9.3	7.8±0.2	*8.5±0.5*	0.9	1.0	1.0	0.8	4	11	14	0.5	1.0	
4901	60.5	52±12	*296±115*	0.8	0.8	0.2	0.2	0.3	0.9	1.3	0.8	0.3	
14E10	79.7	91±3	*117.4±0.7*	0.8	0.8	11	3	18	2	1	3	0.4	
9B8	84.7	76±20	*96±28*	0.8	0.8	0.6	0.6	0.7	0.6	1.3	4	0.4	
13C2	94.4	86±13	*137±5*	0.7	0.7	0.5	0.4	0.6	0.2	0.9	1.1	0.4	
14A9	148.4	219±114	*246±20*	0.8	0.7	0.7	0.5	0.8	0.7	1.6	1.3	6	
6D5	209.9	207±26	*378±22*	0.8	0.7	0.5	0.4	0.6	0.5	3	1.1	5	
1C5	296.4	223±51	*430±173*	0.8	0.8	0.6	0.4	0.6	0.6	1.1	1.1	5	

(57) Abstract: Disclosed herein are methods of treating or reducing incidence of post-traumatic headache and/or at least one secondary symptom associated with post-traumatic headache in a subject comprising administering to the subject a monoclonal antibody that modulates the CGRP pathway. Compositions for use in the disclosed methods are also provided. Antagonist antibody G1 and antibodies derived from G1 directed to CGRP are also described.

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PREVENTING, TREATING, AND REDUCING (PERSISTENT) POST-TRAUMATIC HEADACHE

Cross Reference to Related Applications

This application claims the benefit of priority of U.S. Application Nos. 62/232,343, filed on September 24, 2015, and 62/375,825, filed on August 16, 2016. The contents of the prior applications are hereby incorporated by reference in their entireties.

Background

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide, which belongs to a family of peptides that includes calcitonin, adrenomedullin, adrenomedullin 2 (intermedin), and amylin (Russell et al., Physiol Rev 94:1099-1142, 2014). In humans, two forms of CGRP (α-CGRP and β-CGRP) exist and have similar activities. They vary by three amino acids and exhibit differential distribution. At least two CGRP receptor subtypes may also account for differential activities. CGRP is a neurotransmitter in the central nervous system, and has been shown to be a potent vasodilator in the periphery, where CGRP-containing neurons are closely associated with blood vessels. CGRP-mediated vasodilatation is also associated with neurogenic inflammation, as part of a cascade of events that results in extravasation of plasma and vasodilation of the microvasculature.

Headache is a common complication of traumatic brain injury (TBI), especially so after (repetitive) mild TBI (mTBI) or a mild closed head injury (mCHI). These headaches can be challenging to manage. The headaches can be of high frequency, if not daily. Recognizing the complexity of post-traumatic headaches (PTH), the International Headache Society, in the International Classification of Headache Disorders (ICHD), has a separate classification specific for these headaches (Headache Classification Subcommittee of the International Headache Society, 2004 and the updated edition, 2013). See Riechers et al. (Handbook of Clinical Neurology 128:567-78, 2015). The second edition (ICHD-II) and the 3rd edition (ICHD-3 beta) define PTH as a headache developing within 7 days of the trauma event or emergence from comatose state. These headaches can be defined as acute in the first three months

following injury; however, if they persist beyond this, they are defined as chronic. The severity of the head injury can be further used to subdivide categories of PTH into PTH resulting from mild head injury (Glasgow Coma Scale score (GCS) 13–15, loss of consciousness less than 30 minutes, or other symptoms of concussion) or PTH resulting from severe head injury (GCS less than 13, loss of consciousness greater than 30 minutes, amnesia greater than 48 hours, or abnormal imaging). An additional diagnostic category added to ICHD-II is that of headache due to intracranial hematoma, with epidural and subdural hematomas being the primary hematomas in question. To meet ICHD criteria, these hematomas must be seen on imaging and in the case of epidural hematoma it must develop within 24 hours of the hematoma and resolve within 3 months following surgical intervention, whereas the subdural hematoma headache should develop within 24–72 hours and no specific resolution criteria exist.

Summary

Disclosed herein are anti-CGRP antagonist antibodies and methods of using the same for preventing, treating, or reducing incidence of (persistent) post-traumatic headache. Also disclosed herein are methods of preventing, treating, or reducing incidence of (persistent) post-traumatic headache in a subject comprising administering to the subject a monoclonal antibody that modulates the CGRP pathway.

Methods of preventing, treating, or reducing incidence of at least one secondary symptom associated with (persistent) post-traumatic headache in a subject comprising administering to the subject a monoclonal antibody that modulates the CGRP pathway are also provided. In some embodiments, the amount of the monoclonal antibody administered to the patient can be about 225 mg to about 1000 mg, e.g., about 675 mg or about 900 mg. Accordingly, in some aspects, the methods of preventing, treating, or reducing incidence of (persistent) post-traumatic headache in a subject can comprise administering to the subject a monoclonal antibody that modulates the CGRP pathway, wherein the amount of the monoclonal antibody administered to the patient can be about 225 mg to about 1000 mg, e.g., about 675 mg or about 900 mg. In other aspects, the methods of preventing, treating, or reducing incidence of at least one secondary symptom associated with (persistent) post-traumatic headache in a subject can

comprise administering to the subject a monoclonal antibody that modulates the CGRP pathway are also provided, wherein the amount of the monoclonal antibody administered to the patient can be about 225 mg to about 1000 mg, e.g., about 675 mg or about 900 mg. In one embodiment, the dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for about one year, e.g., two years, three years, four years, or five years.

Suitable administration schedules include, but are not limited to, monthly, quarterly, or a single dose. In some embodiments, the monoclonal antibody can be administered monthly. For example, the monoclonal antibody can be administered monthly for 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more months. In some aspects, the monoclonal antibody can be administered monthly for three or more months. When administered monthly, the dose of the monoclonal antibody administered to the patient can be about 225 mg to about 900 mg.

The monoclonal antibody can be administered as a single dose. When administered as a single dose, the dose of the monoclonal antibody administered to the patient can be about 675 mg to about 1000 mg.

The treating or reducing can comprise reducing the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing a 6-item Headache Impact Test (HIT-6) disability score, improving 12-Item Short Form Health Survey (SF-12) score (Ware et al., Med Care 4:220–233, 1996), reducing Patient Global Impression of Change (PGIC) score (Hurst et al., J Manipulative Physiol Ther 27:26-35, 2004), improving Sport ConCuSSion ASSeSment tool 3 (SCAT-3) score (McCrory et al. British Journal of Sports Medicine 47:263–266, 2013), or any combination thereof. In some

embodiments, the number of monthly headache days can be reduced for at least seven days after a single administration.

In some embodiments, monthly headache hours experienced by the subject after said administering is reduced by 40 or more hours (e.g., 45, 50, 55, 60, 65, 70, 75, 80, or more) from a pre-administration level in the subject. Monthly headache hours may be reduced by more than 60 hours. In some embodiments, monthly headache hours experienced by the subject after said administering are reduced by 25% or more (e.g., 30%, 35%, 40%, 45%, 50%, or more) relative to a pre-administration level in the subject. Monthly headache hours may be reduced by 40% or more. In some embodiments, monthly headache days experienced by the subject after said administering is reduced by three or more days (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more days) from a pre-administration level in the subject. In some embodiments, the number of monthly headache days can be reduced by at least about 50% from a pre-administration level in the subject. Thus, in some aspects, the number of monthly headache days can be reduced by at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, or at least about 90%.

In some embodiments, the administering can be subcutaneous administration. In some embodiments, the administering can be intravenous administration. In some embodiments, the administering can comprise utilizing a pre-filled syringe, pre-filled syringe with a needle safety device, injection pen, or auto-injector comprising a dose of the monoclonal antibody. In some embodiments, the monoclonal antibody can be formulated at a concentration of at least 150 mg/mL. In some embodiments, the monoclonal antibody can be administered in a volume of less than 2 mL, e.g., about 1.5 mL.

In some embodiments, the method further comprises administering to the subject a second agent simultaneously or sequentially with the monoclonal antibody. The second agent can be any of 5-HT1 agonists, triptans, ergot alkaloids, and non-steroidal anti-inflammatory drugs. In some embodiments, the second agent is an agent taken by the subject prophylactically. In some embodiments, monthly use of the second agent by

the subject is decreased by at least about 15%, e.g., at least 16%, 17%, 18%, 20%, 22%, 25%, 28%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or at least about 95%, after administering the monoclonal antibody. In some embodiments, the second agent is a triptan.

In some embodiments, the subject is a human.

The monoclonal antibody can be an anti-CGRP antagonist antibody. In some embodiments, the monoclonal antibody is a human or humanized monoclonal antibody. In some embodiments, the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

Also disclosed are methods of decreasing a number of monthly headache hours experienced by a subject having (persistent) post-traumatic headache. In one embodiment, the method comprises administering to the subject an amount of a monoclonal antibody that modulates the CGRP pathway, wherein the monoclonal antibody is in an amount effective to decrease the number of monthly headache hours by at least 20 (e.g., 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 or more headache hours) after a single dose. In some embodiments, the number of monthly headache hours is reduced by at least about 50 hours. In one embodiment, the method comprises administering to the subject an amount of a monoclonal antibody that modulates the CGRP pathway, wherein the monoclonal antibody is in an amount effective to decrease the number of monthly headache hours by at least 15% (e.g., 20%, 25%, 30%, 35%, 40%, or more) after a single dose. In some embodiments, the number of monthly headache hours is reduced by at least about 30%. In some embodiments, the monoclonal antibody is an anti-CGRP antagonist antibody. In some embodiments, the amount of the monoclonal antibody administered to the patient is about 225 mg to about 1000 mg. In some embodiments, the monoclonal antibody is administered monthly. In some embodiments, the monoclonal antibody is administered as a single dose. In some embodiments, the administering is subcutaneous or intravenous administration.

In some embodiments, the monoclonal antibody is formulated at a concentration of at least 150 mg/mL. In some embodiments, the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL. In some embodiments, the subject is human. In some embodiments, the monoclonal antibody is human or humanized. In some embodiments, the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

Also disclosed are methods of decreasing a number of monthly headache days experienced by a subject having (persistent) post-traumatic headache. In one embodiment, the method comprises administering to the subject an amount of a monoclonal antibody that modulates the CGRP pathway, wherein the monoclonal antibody is in an amount effective to decrease the number of monthly headache days by at least 3 (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more headache days) after a single dose. In some embodiments, the number of monthly headache days is reduced by at least about 6 headache days. In some embodiments, the number of monthly headache days can be reduced by at least about 50% from a pre-administration level in the subject. Thus, in some aspects, the number of monthly headache days can be reduced by at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, or at least about 90%. In some embodiments, the monoclonal antibody is an anti-CGRP antagonist antibody. In some embodiments, the amount of the monoclonal antibody administered to the patient is about 225 mg to about 1000 mg. In some embodiments, the monoclonal antibody is administered monthly. In some embodiments, the monoclonal antibody is administered as a single dose. In some embodiments, the administering is subcutaneous or intravenous administration. In some embodiments, the monoclonal antibody is formulated at a concentration of at least 150 mg/mL. In some embodiments, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL. In some embodiments, the subject is

human. In some embodiments, the monoclonal antibody is human or humanized. In some embodiments, the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

Also disclosed are methods of decreasing use of any acute headache medication in a subject having (persistent) post-traumatic headache, comprising administering to the subject a monoclonal antibody (e.g., anti-CGRP antagonist antibody) that modulates the CGRP pathway, wherein the monoclonal antibody is in an amount effective to decrease monthly use of the anti-headache medication by the subject by at least 15% (e.g., 20%, 25%, 30%, 35%, 40%, or more). In some embodiments, the anti-headache medication is selected from the group consisting of 5-HT1 agonists, triptans, opiates, β-adrenergic antagonists, ergot alkaloids, and non-steroidal anti-inflammatory drugs (NSAIDs). In some embodiments, the anti-headache medication is a triptan. In some embodiments, the amount of the monoclonal antibody administered to the patient is about 225 mg to about 1000 mg, e.g., about 675 mg or about 900 mg. In some embodiments, the monoclonal antibody is administered monthly. In some embodiments, the monoclonal antibody is administered as a single dose. In some embodiments, the administering is subcutaneous or intravenous administration. In some embodiments, the monoclonal antibody is formulated at a concentration of at least 150 mg/mL. In some embodiments, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL. In some embodiments, the subject is human. In some embodiments, the monoclonal antibody is human or humanized. In some embodiments, the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

In one aspect, the invention provides a method of preventing, treating, or reducing incidence of (persistent) post-traumatic headache in a subject comprising administering to the subject a single dose of a monoclonal antibody (e.g., monoclonal anti-CGRP-antagonist antibody) in an amount that modulates the CGRP pathway, wherein the amount of the monoclonal antibody is about 225 mg to about 1000 mg, e.g., about 675 mg or about 900 mg.

In a further embodiment, the invention provides methods for preventing, treating, ameliorating, controlling, reducing incidence of, or delaying the development or progression of (persistent) post-traumatic headache in an individual comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody in combination with at least one additional agent useful for treating the post-traumatic headache. Such additional agents include 5-HT1-like agonists (and agonists acting at other 5-HT1 sites), and non-steroidal anti-inflammatory drugs (NSAIDs).

Examples of 5-HT1 agonists that can be used in combination with an anti-CGRP antibody include a class of compounds known as triptans, such as sumatriptan, zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan, and frovatriptan. Ergot alkaloids and related compounds are also known to have 5-HT agonist activity. Included among these compounds are ergotamine tartrate, ergonovine maleate, and ergoloid mesylates (e.g., dihydroergocornine, dihydroergocristine, dihydroergocryptine, and dihydroergotamine mesylate (DHE 45)).

Examples of NSAIDs that can be used in combination with an anti-CGRP antibody include aspirin, diclofenac, diflusinal, etodolac, fenbufen, fenoprofen, flufenisal, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamic acid, mefenamic acid, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tolmetin or zomepirac, cyclooxygenase-2 (COX-2) inhibitors, celecoxib; rofecoxib; meloxicam; JTE-522; L-745,337; NS398; or a pharmaceutically acceptable salt thereof.

In one embodiment, the anti-CGRP antagonist antibody used in any of the methods described above is any of the antibodies as described herein.

In some embodiments, the anti-CGRP antagonist antibody recognizes a human CGRP. In some embodiments, the anti-CGRP antagonist antibody binds to both human α-CGRP and β-CGRP. In some embodiments, the anti-CGRP antagonist antibody binds human and rat CGRP. In some embodiments, the anti-CGRP antagonist antibody binds the C-terminal fragment having amino acids 25-37 of CGRP. In some embodiments, the anti-CGRP antagonist antibody binds a C-terminal epitope within amino acids 25-37 of CGRP.

In some embodiments, the anti-CGRP antagonist antibody is a monoclonal antibody. In some embodiments, the anti-CGRP antagonist antibody is humanized. In some embodiments, the antibody is human. In some embodiments, the anti-CGRP antagonist antibody is antibody G1 (as described herein). In some embodiments, the anti-CGRP antagonist antibody comprises one or more CDR(s) (such as one, two, three, four, five, or, in some embodiments, all six CDRs) of antibody G1 or variants of G1 shown in Table 6. In still other embodiments, the anti-CGRP antagonist antibody comprises the amino acid sequence of the heavy chain variable region shown in Figure 5 (SEQ ID NO:1) and the amino acid sequence of the light chain variable region shown in Figure 5 (SEQ ID NO:2).

In some embodiments, the antibody comprises a modified constant region, such as a constant region that is immunologically inert (including partially immunologically inert), e.g., does not trigger complement mediated lysis, does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC), does not activate microglia, or having reduced one or more of these activities. In some embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624; PCT Application No. PCT/GB99/01441; and/or UK Patent Application No. 9809951.8. In other embodiments, the antibody comprises a human heavy chain IgG2 constant region comprising the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence). Eur. J. Immunol. (1999) 29:2613-2624. In some embodiments, the heavy chain constant region of the antibody is a human heavy chain IgG1 with any of the following mutations: 1) A327A330P331 to G327S330S331; 2) E233L234L235G236 (SEQ ID NO:48) to P233V234A235 with G236 deleted; 3)

E233L234L235 to P233V234A235; 4) E233L234L235G236A327A330P331 (SEQ ID NO:49) to P233V234A235G327S330S331 (SEQ ID NO:50) with G236 deleted; 5) E233L234L235A327A330P331 (SEQ ID NO:51) to P233V234A235G327S330S331 (SEQ ID NO:50); and 6) N297 to A297 or any other amino acid except N. In some embodiments, the heavy chain constant region of the antibody is a human heavy chain IgG4 with any of the following mutations: E233F234L235G236 (SEQ ID NO:52) to P233V234A235 with G236 deleted; E233F234L235 to P233V234A235; and S228L235 to P228E235.

In still other embodiments, the constant region is aglycosylated for N-linked glycosylation. In some embodiments, the constant region is aglycosylated for N-linked glycosylation by mutating the oligosaccharide attachment residue (such as Asn297) and/or flanking residues that are part of the N-glycosylation recognition sequence in the constant region. In some embodiments, the constant region is aglycosylated for N-linked glycosylation. The constant region may be aglycosylated for N-linked glycosylation enzymatically or by expression in a glycosylation deficient host cell.

The binding affinity (K_D) of an anti-CGRP antagonist antibody to CGRP (such as human α-CGRP as measured by surface plasmon resonance at an appropriate temperature, such as 25 or 37 °C) can be about 0.02 to about 200 nM. In some embodiments, the binding affinity is any of about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, about 60 pM, about 50 pM, about 20 pM, about 15 pM, about 10 pM, about 5 pM, or about 2 pM. In some embodiments, the binding affinity is less than any of about 250 nM, about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, or about 50 pM. In some embodiments, the binding affinity is less than about 50 nM.

The anti-CGRP antagonist antibody may be administered prior to, during and/or after post-traumatic headache. In some embodiments, the anti-CGRP antagonist antibody is administered prior to the attack of post-traumatic headache (e.g., after trauma or injury to the head and/or neck). Administration of an anti-CGRP antagonist antibody can be by any means known in the art, including: orally, intravenously, subcutaneously, intraarterially, intramuscularly, intranasally (e.g., with or without

inhalation), intracardially, intraspinally, intrathoracically, intraperitoneally, intraventricularly, sublingually, transdermally, and/or via inhalation. Administration may be systemic, e.g., intravenously, or localized. In some embodiments, an initial dose and one or more additional doses are administered the same way, i.e., subcutaneously or intravenously. In some embodiments, the one or more additional doses are administered in a different way than the initial dose, i.e., the initial dose may be administered intravenously and the one or more additional doses may be administered subcutaneously.

In some embodiments, the anti-CGRP antagonist antibody may be administered in conjunction with another agent, such as another agent for treating post-traumatic headache.

In another aspect, the invention provides use of an anti-CGRP antagonist antibody for the manufacture of a medicament for use in any of the methods described herein, for example, for preventing, treating, or reducing (persistent) post-traumatic headache.

In another aspect, the invention provides a pharmaceutical composition for preventing, treating, or reducing post-traumatic headache comprising an effective amount of an anti-CGRP antagonist antibody, in combination with one or more pharmaceutically acceptable excipients.

In another aspect, the invention provides a kit for use in any of the methods described herein. In some embodiments, the kit comprises a container, a composition comprising an anti-CGRP antagonist antibody described herein, in combination with a pharmaceutically acceptable carrier, and instructions for using the composition in any of the methods described herein.

The present invention also provides anti-CGRP antagonist antibodies and polypeptides derived from antibody G1 or its variants shown in Table 6. Accordingly, in one aspect, the invention provides an antibody G1 (interchangeably termed "G1" and “TEV-48125”) that is produced by expression vectors having ATCC Accession Nos. PTA-6866 and PTA-6867. For example, in one embodiment is an antibody comprising a heavy chain produced by the expression vector with ATCC Accession No. PTA-6867.

In a further embodiment is an antibody comprising a light chain produced by the expression vector with ATCC Accession No. PTA-6866. The amino acid sequences of the heavy chain and light chain variable regions of G1 are shown in Figure 5. The complementarity determining region (CDR) portions of antibody G1 (including Chothia and Kabat CDRs) are also shown in Figure 5. It is understood that reference to any part of or entire region of G1 encompasses sequences produced by the expression vectors having ATCC Accession Nos. PTA-6866 and PTA-6867, and/or the sequences depicted in Figure 5. In some embodiments, the invention also provides antibody variants of G1 with amino acid sequences depicted in Table 6.

In one aspect, the invention provides an antibody comprising a V_H domain that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% at least 98%, at least 99% or 100% identical in amino acid sequence to SEQ ID NO:1.

In another aspect, the invention provides an antibody comprising a V_L domain that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% at least 98%, at least 99% or 100% identical in amino acid sequence to SEQ ID NO:2.

In another aspect, the invention provides an antibody comprising a fragment or a region of the antibody G1 or its variants shown in Table 6. In one embodiment, the fragment is a light chain of the antibody G1. In another embodiment, the fragment is a heavy chain of the antibody G1. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain of the antibody G1. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain shown in Figure 5. In yet another embodiment, the fragment contains one or more CDRs from a light chain and/or a heavy chain of the antibody G1.

In another aspect, the invention provides polypeptides (which may or may not be an antibody) comprising a V_H CDR3 as set forth in SEQ ID NO:5, or a sequence that

differs from SEQ ID NO:5 by 1, 2, 3, 4, or 5 amino acid substitutions. In a particular embodiment, such amino acid substitutions are conservative substitutions.

In another aspect, the invention provides polypeptides (which may or may not be an antibody) comprising a V_L CDR3 as set forth in SEQ ID NO:8, or a sequence that differs from SEQ ID NO:8 by 1, 2, 3, 4, or 5 amino acid substitutions. In a particular embodiment, such amino acid substitutions are conservative substitutions.

In another aspect, the invention provides polypeptides (which may or may not be an antibody) comprising any one or more of the following: a) one or more CDR(s) of antibody G1 or its variants shown in Table 6; b) CDR H3 from the heavy chain of antibody G1 or its variants shown in Table 6; c) CDR L3 from the light chain of antibody G1 or its variants shown in Table 6; d) three CDRs from the light chain of antibody G1 or its variants shown in Table 6; e) three CDRs from the heavy chain of antibody G1 or its variants shown in Table 6; f) three CDRs from the light chain and three CDRs from the heavy chain of antibody G1 or its variants shown in Table 6. In some embodiments, the invention further provides polypeptides (which may or may not be an antibody) comprising any one or more of the following: a) one or more (one, two, three, four, five, or six) CDR(s) derived from antibody G1 or its variants shown in Table 6; b) a CDR derived from CDR H3 from the heavy chain of antibody G1; and/or c) a CDR derived from CDR L3 from the light chain of antibody G1. In some embodiments, the CDR is a CDR shown in Figure 5. In some embodiments, the one or more CDRs derived from antibody G1 or its variants shown in Table 6 are at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to at least one, at least two, at least three, at least four, at least five, or at least six CDRs of G1 or its variants.

In some embodiments, the CDR is a Kabat CDR. In other embodiments, the CDR is a Chothia CDR. In other embodiments, the CDR is a combination of a Kabat and a Chothia CDR (also termed "combined CDR" or "extended CDR"). In other words,

for any given embodiment containing more than one CDR, the CDRs may be any of Kabat, Chothia, and/or combined.

In some embodiments, the polypeptide (such as an antibody) comprises the amino acid sequence of KASKXaaVXaaTYVS (SEQ ID NO:53), wherein Xaa at position 5 is R, W, G, L, or N; and wherein Xaa at position 7 is T, A, D, G, R, S, W, or V. In some embodiments, the amino acid sequence of KASKXaaVXaaTYVS (SEQ ID NO:53) is CDR1 of an antibody light chain.

In some embodiments, the polypeptide (such as an antibody) comprises the amino acid sequence of XaaXaaSNRYXaa (SEQ ID NO:54), wherein Xaa at position 1 is G or A; wherein Xaa at position 2 is A or H; and wherein Xaa at position 7 is L, T, I, or S. In some embodiments, the amino acid sequence of XaaXaaSNRYXaa (SEQ ID NO:54) is CDR2 of an antibody light chain.

In some embodiments, the polypeptide (such as an antibody) comprises the amino acid sequence of EIRSXaaSDXaaXaaATXaaYAXaaAVKG (SEQ ID NO:55), wherein Xaa at position 5 is E, R, K, Q, or N; wherein Xaa at position 8 is A, G, N, E, H, S, L, R, C, F, Y, V, D, or P; wherein Xaa at position 9 is S, G, T, Y, C, E, L, A, P, I, N, R, V, D, or M; wherein Xaa at position 12 is H or F; wherein Xaa at position 15 is E or D. In some embodiments, the amino acid sequence of EIRSXaaSDXaaXaaATXaaYAXaaAVKG (SEQ ID NO:55) is CDR2 of an antibody heavy chain.

In some embodiments, the polypeptide (such as an antibody) comprises the amino acid sequence of SEQ ID NO:1, wherein amino acid residue at position 99 of SEQ ID NO:1 is L or is substituted by A, N, S, T, V, or R; and wherein amino acid residues at position 100 of SEQ ID NO:1 is A or is substituted by L, R, S, V, Y, C, G, T, K, or P.

In some embodiments, the antibody is a human antibody. In other embodiments, the antibody a humanized antibody. In some embodiments, the antibody is monoclonal. In some embodiments, the antibody (or polypeptide) is isolated. In some embodiments, the antibody (or polypeptide) is substantially pure.

The heavy chain constant region of the antibodies may be from any types of constant region, such as IgG, IgM, IgD, IgA, and IgE; and any isotypes, such as IgG1, IgG2, IgG3, and IgG4.

In some embodiments, the antibody comprises a modified constant region as described herein.

In another aspect, the invention provides a polynucleotide (which may be isolated) comprising a polynucleotide encoding a fragment or a region of the antibody G1 or its variants shown in Table 6. In one embodiment, the fragment is a light chain of the antibody G1. In another embodiment, the fragment is a heavy chain of the antibody G1. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain of the antibody G1. In yet another embodiment, the fragment contains one or more (i.e., one, two, three, four, five, or six) complementarity determining regions (CDRs) from a light chain and/or a heavy chain of the antibody G1.

In another aspect, the invention provides a polynucleotide (which may be isolated) comprising a polynucleotide that encodes for antibody G1 or its variants shown in Table 6. In some embodiments, the polynucleotide comprises either or both of the polynucleotides shown in SEQ ID NO:9 and SEQ ID NO:10.

In another aspect, the invention provides polynucleotides encoding any of the antibodies (including antibody fragments) or polypeptides described herein.

In another aspect, the invention provides vectors (including expression and cloning vectors) and host cells comprising any of the polynucleotide disclosed herein. In some embodiments, the vector is pDb.CGRP.hFcGl having ATCC No. PTA-6867. In other embodiments, the vector is pEb.CGRP.hKGl having ATCC No. PTA-6866.

In another aspect, the invention provides a host cell comprising a polynucleotide encoding any of the antibodies described herein.

In another aspect, the invention provides a complex of CGRP bound by any of the antibodies or polypeptides described herein. In some embodiments, the antibody is antibody G1 or its variants shown in Table 6.

In another aspect, the invention provides a pharmaceutical composition comprising an effective amount of any of the polypeptides (including antibodies, such as an antibody comprising one or more CDRs of antibody G1) or polynucleotides described herein, and a pharmaceutically acceptable excipient.

In another aspect, the invention provides a method of generating antibody G1 comprising culturing a host cell or progeny thereof under conditions that allow production of antibody G1, wherein the host cell comprises an expression vector that encodes for antibody G1; and, in some embodiments, purifying the antibody G1. In some embodiments, the expression vector comprises one or both of the polynucleotide sequences shown in SEQ ID NO:9 and SEQ ID NO:10.

In another aspect, the invention provides methods of generating any of the antibodies or polypeptides described herein by expressing one or more polynucleotides encoding the antibody (which may be separately expressed as a single light or heavy chain, or both a light and a heavy chain are expressed from one vector) or the polypeptide in a suitable cell, generally followed by recovering and/or isolating the antibody or polypeptides of interest.

The anti-CGRP antagonist antibody and polypeptides, and polynucleotides encoding the antibodies and polypeptides of the present invention may be used for preventing, treating, preventing, ameliorating, controlling, or reducing incidence of diseases associated with abnormal function of CGRP, such as post-traumatic headache and other conditions that may be prevented, treated, or prevented by antagonizing CGRP activity.

In another aspect, the invention provides kits and compositions comprising any one or more of the compositions described herein. These kits, generally in suitable packaging and provided with appropriate instructions, are useful for any of the methods described herein.

In one aspect, the invention provides a composition for use in accordance with any of the methods described herein.

In one aspect, the invention provides a composition for use in decreasing a number of monthly headache hours experienced by a subject. In one embodiment, the

use comprises administering to the subject an amount of a monoclonal antibody that modulates the CGRP pathway, wherein the monoclonal antibody is in an amount effective to decrease the number of monthly headache hours by at least 20 (e.g., 25, 30, 35, 40, 45, 50, 55, 60, 65, 70 or more headache hours) after a single dose. In some embodiments, the number of monthly headache hours is reduced by at least about 50 hours. In one embodiment, the use comprises administering to the subject an amount of a monoclonal antibody that modulates the CGRP pathway, wherein the monoclonal antibody is in an amount effective to decrease the number of monthly headache hours by at least 15% (e.g., 20%, 25%, 30%, 35%, 40%, or more) after a single dose. In some embodiments, the number of monthly headache hours is reduced by at least about 30%. In some embodiments, the monoclonal antibody is an anti-CGRP antagonist antibody. In some embodiments, the amount of the monoclonal antibody administered to the patient is about 675 mg to about 1000 mg. In some embodiments, the monoclonal antibody is administered monthly. In some embodiments, the monoclonal antibody is administered as a single dose. In some embodiments, the administering is subcutaneous or intravenous administration. In some embodiments, the monoclonal antibody is formulated at a concentration of at least 150 mg/mL. In some embodiments, wherein the monoclonal antibody is administered in a volume of less than 2 mL. In some embodiments, the subject is human. In some embodiments, the monoclonal antibody is human or humanized. In some embodiments, the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

In one aspect, the invention provides a composition for use in decreasing a number of monthly headache days experienced by a subject. In one embodiment, the use comprises administering to the subject an amount of a monoclonal antibody that modulates the CGRP pathway, wherein the monoclonal antibody is in an amount effective to decrease the number of monthly headache days by at least 3 (e.g., 3, 4, 5,

6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more headache days) after a single dose. In some embodiments, the number of monthly headache days is reduced by at least about 6 headache days. In some embodiments, the monoclonal antibody is an anti-CGRP antagonist antibody. In some embodiments, the amount of the monoclonal antibody administered to the patient is about 675 mg to about 1000 mg. In some embodiments, the monoclonal antibody is administered monthly. In some embodiments, the monoclonal antibody is administered as a single dose. In some embodiments, the administering is subcutaneous or intravenous administration. In some embodiments, the monoclonal antibody is formulated at a concentration of at least 150 mg/mL. In some embodiments, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL. In some embodiments, the subject is human. In some embodiments, the monoclonal antibody is human or humanized. In some embodiments, the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

In one aspect, the invention provides a composition for use in decreasing use of any acute headache medication in a subject, comprising administering to the subject a monoclonal antibody (e.g., anti-CGRP antagonist antibody) that modulates the CGRP pathway, wherein the monoclonal antibody is in an amount effective to decrease monthly use of the acute headache medication by the subject by at least 15% (e.g., 20%, 25%, 30%, 35%, 40%, or more). In some embodiments, the anti-headache medication is selected from the group consisting of 5-HT1 agonists, triptans, opiates, β-adrenergic antagonists, ergot alkaloids, and non-steroidal anti-inflammatory drugs (NSAIDs). In some embodiments, the anti-headache medication is a triptan. In some embodiments, the amount of the monoclonal antibody administered to the patient is about 675 mg to about 1000 mg. In some embodiments, the monoclonal antibody is administered monthly. In some embodiments, the monoclonal antibody is administered as a single dose. In some embodiments, the administering is subcutaneous or

intravenous administration. In some embodiments, the monoclonal antibody is formulated at a concentration of at least 150 mg/mL. In some embodiments, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL. In some embodiments, the subject is human. In some embodiments, the monoclonal antibody is human or humanized. In some embodiments, the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

In one aspect, the invention provides a composition for use in of preventing, treating, or reducing incidence of post-traumatic headache in a subject comprising administering to the subject a single dose of a monoclonal antibody (e.g., monoclonal anti-CGRP-antagonist antibody) in an amount that modulates the CGRP pathway, wherein the amount of the monoclonal antibody administered to the patient is about 675 mg to about 1000 mg.

Brief Description of the Drawings

Figure 1 is a table showing binding affinities of 12 murine antibodies for different alanine substituted human α-CGRP fragments. Binding affinities were measured at 25°C using Biacore by flowing Fabs across CGRPs on the chip. The boxed values represent the loss in affinity of alanine mutants relative to parental fragment, 25-37 (italic), except K35A, which was derived from a 19-37 parent. "a" indicates affinities for 19-37 and 25-37 fragments are the mean average ± standard deviation of two independent measurements on different sensor chips. "b" indicates these interactions deviated from a simple bimolecular interaction model due to a biphasic offrate, so their affinities were determined using a conformational change model. Grey-scale key: white (1.0) indicates parental affinity; light grey (less than 0.5) indicates higher affinity than parent; dark grey (more than 2) indicates lower affinity than parent; and black indicates that no binding was detected.

Figures 2A and 2B show the effect of administering CGRP 8-37 (400 nmol/kg), antibody 4901 (25 mg/kg), and antibody 7D11 (25 mg/kg) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds. CGRP 8-37 was administered intravenously (iv) 3-5 min before electrical pulse stimulation. Antibodies were administered intraperitoneal (IP) 72 hours before electrical pulse stimulation. Each point in the graphs represents AUC of one rat treated under the conditions as indicated. Each line in the graphs represents average AUC of rats treated under the condition as indicated. AUC (area under the curve) equals to Δflux x Δtime. "Δflux" represents the change of flux units after the electrical pulse stimulation; and "Δtime" represents the time period taken for the blood cell flux level to return to the level before the electrical pulse stimulation.

Figure 3 shows the effect of administering different dosage of antibody 4901 (25 mg/kg, 5 mg/kg, 2.5 mg/kg, or 1 mg/kg) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds. Antibodies were administered intravenously (IV) 24 hours before electrical pulse stimulation. Each point in the graph represents AUC of one rat treated under the conditions as indicated. The line in the graph represents average AUC of rats treated under the condition as indicated.

Figures 4A and 4B show the effect of administering antibody 4901 (1 mg/kg or 10 mg/kg, i.v.), antibody 7E9 (10 mg/kg, i.v.), and antibody 8B6 (10 mg/kg, i.v.) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds. Antibodies were administered intravenously (i.v.) followed by electrical pulse stimulation at 30 min, 60 min, 90 min, and 120 min after antibody administration. Y axis represents percent of AUC as compared to level of AUC when no antibody was administered (time 0). X axis represents time (minutes) period between the administration of antibodies and electrical pulse stimulation. "*" indicates $P < 0.05$, and "**" indicates $P < 0.01$, as compared to time 0. Data were analyzed using one-way ANOVA with a Dunnett's Multiple comparison test.

Figure 5 shows the amino acid sequence of the heavy chain variable region (SEQ ID NO:1) and light chain variable region (SEQ ID NO:2) of antibody G1. The

Kabat CDRs are in bold text, and the Chothia CDRs are underlined. The amino acid residues for the heavy chain and light chain variable region are numbered sequentially.

Figure 6 shows epitope mapping of antibody G1 by peptide competition using Biacore. N-biotinylated human α-CGRP was captured on SA sensor chip. G1 Fab (50 nM) in the absence of a competing peptide or pre-incubated for 1 hour with 10 μM of a competing peptide was flowed onto the chip. Binding of G1 Fab to the human α-CGRP on the chip was measured. Y axis represents percentage of binding blocked by the presence of the competing peptide compared with the binding in the absence of the competing peptide.

Figure 7 shows the effect of administering antibody G1 (1 mg/kg or 10 mg/kg, i.v.) or vehicle (PBS, 0.01% Tween 20) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds. Antibody G1 or vehicle was administered intravenously (i.v.) followed by nerve electrical pulse stimulation at 30 min, 60 min, 90 min, and 120 min after antibody administration. Y axis represents percent of AUC as compared to level of AUC when no antibody or vehicle (defined as 100%) was administered (time 0). X axis represents time (minutes) period between the administration of antibodies and electrical pulse stimulation. "*" indicates $P < 0.05$, and "**" indicates $P < 0.01$, as compared to vehicle. Data were analyzed using two-way ANOVA and Bonferroni post tests.

Figure 8A shows the effect of administering antibody G1 (1 mg/kg, 3 mg/kg or 10 mg/kg, i.v.) or vehicle (PBS, 0.01% Tween 20) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds 24 hours after dosing. Antibody G1 or vehicle was administered intravenously (i.v.) 24 hours before nerve electrical pulse stimulation. Y axis represents total area under curve (change in blood cell flux multiplied by the change in time from stimulation until flux returns to baseline, AUC). X axis represents varying doses of antibody G1. "*" indicates $P < 0.05$, and "**" indicates $P < 0.01$, as compared to vehicle. Data were analyzed using one-way ANOVA and Dunn's multiple comparison test.

Figure 8B shows the effect of administering antibody G1 (0.3 mg/kg, 1 mg/kg, 3 mg/kg or 10 mg/kg, i.v.) or vehicle (PBS, 0.01% Tween 20) on skin blood flow measured

as blood cell flux after electrical pulse stimulation for 30 seconds 7 days after dosing. Antibody G1 or vehicle was administered intravenously (i.v.) 7 days before nerve electrical pulse stimulation. Y axis represents total AUC. X axis represents varying doses of antibody G1. "**" indicates $P < 0.01$, and "***" indicates $P < 0.001$, as compared to vehicle. Data were analyzed using one-way ANOVA and Dunn's multiple comparison test.

Figure 8C is a curve fit analysis of the data from Figures 8A and 8B. Antibody G1 or vehicle was administered intravenously (i.v.) either 24 hours or 7 days before nerve electrical pulse stimulation. Y axis represents total AUC. X axis represents varying doses of antibody G1 in "mg/kg" on a logarithmic scale to determine EC_{50}.

Figure 9 shows the effect of antibody mu7E9 (10 mg/kg), BIBN4096BS or vehicle (PBS, 0.01% Tween 20) on the change in diameter of the middle meningeal artery after electrical field stimulation. Antibody mu7E9, BIBN4096BS or vehicle were administered intravenously (i.v.) at time point 0 minutes after a baseline response to electrical stimulation was established. Y axis represents change in diameter of the middle meningeal artery after electrical field stimulation. Resting diameter corresponds to 0%. X axis represents time (minutes) of electrical pulse stimulation. "*" indicates $P < 0.05$, and "**" indicates $P < 0.01$, as compared to vehicle. Data were analyzed using one-way ANOVA and Dunett's multiple comparison test.

Figure 10 shows the effect of varying doses of antibody G1 (1 mg/kg, 3 mg/kg or 10 mg/kg, i.v.) or vehicle (PBS, 0.01% Tween 20) on the change in diameter of the middle meningeal artery after electrical field stimulation. Antibody G1 or vehicle was administered intravenously (i.v.) 7 days before electrical field stimulation. Y axis represents change in diameter of the middle meningeal artery. Resting diameter corresponds to 0%. X axis represents stimulation voltage. "*" indicates $P < 0.05$, "**" indicates $P < 0.01$, and "***" indicates $P < 0.001$, as compared to vehicle. Data were analyzed using two-way ANOVA and Bonferroni posttests.

Figure 11A shows the effect of antibody mu4901 (10 mg/kg) or vehicle (PBS, 0.01% Tween 20), administered intravenously (i.v.) 24 hours prior, on the decrease in core temperature induced by subcutaneous injection of naloxone (1 mg/kg) in morphine

addicted rats. The Y axis represents temperature difference from baseline. The X axis represents time measured from the point of naloxone injection.

Figure 11B shows the effect of antibody mu4901 (10 mg/kg) or vehicle (PBS, 0.01% Tween 20), administered intravenously (i.v.) 24 hours prior, on the increase in tail surface temperature induced by subcutaneous injection of naloxone (1 mg/kg) in morphine addicted rats. The Y axis represents temperature difference from baseline. The X axis represents time measured from the point of naloxone injection.

Figures 12A and 12B are two bar graphs that show the effect of mCHI on (12A) vertical exploratory activity and (12B) novel object recognition. Data are mean + SEM (n=8) *p<0.05 vs sham.

Figures 13A to 13D are four line graphs that show cephalic (13A and 13C) and hindpaw (13B and 13D) response threshold and cumulative nociceptive score to mechanical stimulation of over 14 days following mCHI. Data are mean + SEM (n=6). *p<0.05 vs sham.

Figures 14A to 14D are four line graphs that show the effect of acute sumatriptan (Figures 14A and 14B) or chronic murine anti-CGRP antibody (Figures 14C and 14D) treatment on response threshold and cumulative nociceptive score to mechanical stimulation following mCHI. Data are mean + SEM (n=6-8). *p<0.05 sham vs mCHI, #p<0.05 mCHI vs. mCHI + Suma +p<0.05 control IgG vs anti-CGRP mAb.

Figures 15A and 15B show treatments protocol for conditioned place preference during conditioning day (Figure 15A) and that sumatriptan produces CPP in animals at 7 days post mCHI but not in sham animals (Figure 15B). Data are mean + SEM (n=8). **p<0.001 saline paired vs. sumatriptan paired.

Figures 16A to 16D are four bar graphs that show the effect of GTN administration at Day 15 and Day 30 post mCHI on cephalic mechanical hypersensitivity. Data are mean + SEM (n=8) *p<0.05, **p<0.01 ***p<0.001 vs sham.

Figures 17A to 17D are four bar graphs that show the effect of GTN administration at Day 15 and Day 30 post mCHI on hindpaw mechanical hypersensitivity. Data are mean + SEM (n=8) *p<0.05 vs sham.

Figures 18A to 18D are four bar graphs that show the effect of acute sumatriptan treatment or chronic administration of anti-CGRP mAb and their control treatments on the % decrease in response thresholds (Figures 18A and 18C) or % increase in nociceptive scores (Figures 18B and 18D) at 4 h post-GTN compared to corresponding pre-GTN Day 14 values. Data are mean + SEM (n=8) *p<0.05, **p<0.01, ***p<0.001 vs GTN + Veh or GTN + IgG.

Figures 19A and 19B show treatments protocol for conditioned place aversion during conditioning day (Figure 19A) and that GTN produced placed aversion in the IgG injected mCHI animals on Day 14 post-injury but not in the mCHI animals treated with the anti-CGRP mAb (Figure 19B). Data are mean + SEM (n=8). *p<0.05 pre-GTN-paired chamber vs GTN-paired chamber.

DETAILED DESCRIPTION

In some aspects, the invention disclosed herein provides methods for preventing, treating, and/or reducing post-traumatic headache in an individual by administering to the individual a therapeutically effective amount of an anti-CGRP antagonist antibody.

In some aspects, the invention disclosed herein also provides anti-CGRP antagonist antibodies and polypeptides derived from G1 or its variants shown in Table 6. In some embodiments, the invention also provides methods of making and using these antibodies and polypeptides.

General Techniques

The practice of the various aspects of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook

(J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

Definitions

As used herein, "about" when used in reference to numerical ranges, cutoffs, or specific values is used to indicate that the recited values may vary by up to as much as 10% from the listed value. Thus, the term "about" is used to encompass variations of ± 10% or less, variations of ± 5% or less, variations of ± 1% or less, variations of ± 0.5% or less, or variations of ± 0.1% or less from the specified value.

An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv), single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (such as domain antibodies), and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. An antibody

includes an antibody of any class, such as IgG, IgA, or IgM (or sub-class thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its 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, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature, 256:495, or may be made by recombinant DNA methods such as described in U.S. Patent No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., 1990, Nature, 348:552-554, for example.

As used herein, "humanized" antibodies refer to forms of non-human (e.g., murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins

(recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and, biological activity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs “derived from” one or more CDRs from the original antibody.

As used herein, “human antibody” means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies known in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., 1996, Nature Biotechnology, 14:309-314; Sheets et al., 1998, PNAS, (USA) 95:6157-6162; Hoogenboom and Winter, 1991, J. Mol. Biol., 227:381; Marks et al., 1991, J. Mol. Biol., 222:581). Human antibodies can also be made by introducing human immunoglobulin

loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized in vitro). See, e.g., Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., 1991, J. Immunol., 147 (1):86-95; and U.S. Patent No. 5,750,373.

As used herein, the term “calcitonin gene-related peptide” and “CGRP” refers to any form of calcitonin gene-related peptide and variants thereof that retain at least part of the activity of CGRP. For example, CGRP may be α-CGRP or β-CGRP. As used herein, CGRP includes all mammalian species of native sequence CGRP, e.g., human, canine, feline, equine, and bovine.

As used herein, an “anti-CGRP antagonist antibody” (interchangeably termed "anti-CGRP antibody") refers to an antibody that is able to bind to CGRP and inhibit CGRP biological activity and/or downstream pathway(s) mediated by CGRP signaling. An anti-CGRP antagonist antibody encompasses antibodies that modulate, block, antagonize, suppress or reduce (including significantly) CGRP biological activity, or otherwise antagonize the CGRP pathway, including downstream pathways mediated by CGRP signaling, such as receptor binding and/or elicitation of a cellular response to CGRP. For purpose of the present invention, it will be explicitly understood that the term “anti-CGRP antagonist antibody” encompasses all the previously identified terms, titles, and functional states and characteristics whereby CGRP itself, CGRP biological activity (including but not limited to its ability to mediate any aspect of headache), or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree. In some embodiments, an anti-CGRP antagonist antibody binds CGRP and prevents CGRP binding to a CGRP receptor. In other embodiments, an anti-CGRP antibody binds CGRP and prevents activation of a CGRP receptor. Examples of anti-CGRP antagonist antibodies are provided herein.

As used herein, the terms "G1," "antibody G1," and "TEV-48125" are used interchangeably to refer to an anti-CGRP antagonist antibody produced by expression vectors having deposit numbers of ATCC PTA-6867 and ATCC PTA-6866. The amino acid sequence of the heavy chain and light chain variable regions are shown in Figure 5. The CDR portions of antibody G1 (including Chothia and Kabat CDRs) are diagrammatically depicted in Figure 5. The polynucleotides encoding the heavy and light chain variable regions are shown in SEQ ID NO:9 and SEQ ID NO:10. The characterization of G1 is described in the Examples.

The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art. It is understood that, because the polypeptides of this invention are based upon an antibody, the polypeptides can occur as single chains or associated chains.

"Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, "caps", substitution of one or more of the

naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), (O)NR_2 ("amidate"), P(O)R, P(O)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

As used herein, "post-traumatic headache" is a headache attributed to trauma or injury to the head and/or neck, as further described in The International Classification of

Headache Disorders, 3rd edition (beta version), Cephalalgia, 33(9): 629-808 (2013). For example, post-traumatic headaches can resemble tension-type headache or migraine. Consequently, their diagnosis is largely dependent on the close temporal relation between the trauma or injury and headache onset. Consistently with those of ICHD-II, the diagnostic criteria of ICHD-3 beta for all subtypes require that headache must be reported to have developed within 7 days of trauma or injury, or within 7 days after regaining consciousness and/or the ability to sense and report pain when these have been lost following trauma or injury. Although this 7-day interval is somewhat arbitrary, and although some experts argue that headache may develop after a longer interval in a minority of patients, there is not enough evidence at this time to change this requirement.

Skilled practitioners will be readily able to recognize a subject with a post-traumatic headache. For example, traumatic injury to the head has occurred, and a headache is reported to have developed within seven days after one of the following: the injury to the head, regaining of consciousness following the injury to the head, or discontinuation of medication(s) that impair ability to sense or report headache following the injury to the head. In some cases, the headache has resolved within 3 months after the injury to the head, or the headache has not yet resolved but 3 months have not yet passed since the injury to the head.

Diagnostic criteria for acute headaches attributed to traumatic injury to the head, which can include headaches of less than 3 months' duration caused by traumatic injury to the head, can include:

A. Any headache fulfilling criteria C and D

B. Traumatic injury to the head has occurred

C. Headache is reported to have developed within 7 days after one of the following:

1. the injury to the head
2. regaining of consciousness following the injury to the head
3. discontinuation of medication(s) that impair ability to sense or report headache following the injury to the head

D. Either of the following:

1. headache has resolved within 3 months after the injury to the head

2. headache has not yet resolved but 3 months have not yet passed since the injury to the head

E. Not better accounted for by another ICHD-3 diagnosis.

Diagnostic criteria for acute headaches attributed to moderate or severe traumatic injury to the head can include injury to the head associated with at least one of the following:

1. loss of consciousness for >30 minutes

2. Glasgow Coma Scale (GCS) score <13

3. post-traumatic amnesia1 lasting >24 hours

4. alteration in level of awareness for >24 hours

5. imaging evidence of a traumatic head injury such as intracranial haemorrhage and/or brain contusion.

Diagnostic criteria for post-traumatic headache attributed to mild traumatic injury to the head can include injury to the head fulfilling both of the following:

1. associated with none of the following:

a) loss of consciousness for >30 minutes

b) Glasgow Coma Scale (GCS) score <13

c) post-traumatic amnesia lasting >24 hours

d) altered level of awareness for >24 hours

e) imaging evidence of a traumatic head injury such as intracranial haemorrhage and/or brain contusion

2. associated, immediately following the head injury, with one or more of the following symptoms and/or signs:

a) transient confusion, disorientation, or impaired consciousness

b) loss of memory for events immediately before or after the head injury

c) two or more other symptoms suggestive of mild traumatic brain injury: nausea, vomiting, visual disturbances, dizziness and/or vertigo, impaired memory and/or concentration.

Persistent post-traumatic headache attributed to traumatic injury to the head is a headache of greater than three months' duration caused by traumatic injury to the head and headache is reported to have developed within 7 days after one of the following: the injury to the head, regaining of consciousness following the injury to the head, or discontinuation of medication(s) that impair ability to sense or report headache following the injury to the head. In some cases, the headache persists for greater than three months after the injury to the head.

Diagnostic criteria for persistent headache attributed to moderate or severe traumatic injury to the head can include injury to the head associated with at least one of the following:

1. loss of consciousness for >30 minutes
2. Glasgow Coma Scale (GCS) score <13
3. post-traumatic amnesia1 lasting >24 hours
4. alteration in level of awareness for >24 hours
5. imaging evidence of a traumatic head injury such as intracranial haemorrhage and/or brain contusion.

Diagnostic criteria for persistent post-traumatic headache attributed to mild traumatic injury to the head can include injury to the head fulfilling both of the following:

1. associated with none of the following:
    a) loss of consciousness for >30 minutes
    b) Glasgow Coma Scale (GCS) score <13
    c) post-traumatic amnesia lasting >24 hours
    d) altered level of awareness for >24 hours
    e) imaging evidence of a traumatic head injury such as intracranial haemorrhage and/or brain contusion
2. associated, immediately following the head injury, with one or more of the following symptoms and/or signs:
    a) transient confusion, disorientation, or impaired consciousness
    b) loss of memory for events immediately before or after the head injury

c) two or more other symptoms suggestive of mild traumatic brain injury: nausea, vomiting, visual disturbances, dizziness and/or vertigo, impaired memory and/or concentration.

Traumatic injury to the head is defined as a structural or functional injury resulting from the action of external forces on the head. These include striking the head with or the head striking an object, penetration of the head by a foreign body, forces generated from blasts or explosions, and other forces yet to be defined.

A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al., Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda MD)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani et al (1997) J. Molec. Biol. 273:927-948)). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

A "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

An epitope that "preferentially binds" or "specifically binds" (used interchangeably herein) to an antibody or a polypeptide is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it

binds with greater affinity, avidity, more readily, and/or with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a CGRP epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other CGRP epitopes or non-CGRP epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

As used herein, “substantially pure” refers to material which is at least 50% pure (i.e., free from contaminants), more preferably at least 90% pure, more preferably at least 95% pure, more preferably at least 98% pure, and more preferably at least 99% pure.

A “host cell” includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s) of this invention.

The term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3.

As used herein, "Fc receptor" and “FcR” describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover,

a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, 1991, Ann. Rev. Immunol., 9:457-92; Capel et al., 1994, Immunomethods, 4:25-34; and de Haas et al., 1995, J. Lab. Clin. Med., 126:330-41. "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., 1976, J. Immunol., 117:587; and Kim et al., 1994, J. Immunol., 24:249).

"Complement dependent cytotoxicity" and "CDC" refer to the lysing of a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g., an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., J. Immunol. Methods, 202:163 (1996), may be performed.

A "functional Fc region" possesses at least one effector function of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down-regulation of cell surface receptors (e.g., B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g., an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc

region of a parent polypeptide, e.g., from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% sequence identity therewith, more preferably at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity therewith.

As used herein "antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g., natural killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC activity of a molecule of interest can be assessed using an in vitro ADCC assay, such as that described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in an animal model such as that disclosed in Clynes et al., 1998, PNAS (USA), 95:652-656.

As used herein, "preventing" is an approach to stop (persistent) PTH from occurring or existing in a subject, who does not already have (persistent) PTH. As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: improvement in any aspect of a post-traumatic headache including lessening severity, alleviation of pain intensity, and other associated symptoms, reducing frequency of recurrence, increasing the quality of life of those suffering from the post-traumatic headache, and decreasing dose of other medications required to treat the post-traumatic headache.

"Reducing incidence" of post-traumatic headache means any of reducing severity (which can include reducing need for and/or amount of (e.g., exposure to) other drugs and/or therapies generally used for this condition, including, for example, ergotamine,

dihydroergotamine, or triptans), duration, and/or frequency (including, for example, delaying or increasing time to next episodic attack in an individual). As is understood by those skilled in the art, individuals may vary in terms of their response to treatment, and, as such, for example, a "method of reducing incidence of post-traumatic headache in an individual" reflects administering the anti-CGRP antagonist antibody based on a reasonable expectation that such administration may likely cause such a reduction in incidence in that particular individual.

"Ameliorating" post-traumatic headache or one or more symptoms of post-traumatic headache means a lessening or improvement of one or more symptoms of post-traumatic headache as compared to not administering an anti-CGRP antagonist antibody. "Ameliorating" also includes shortening or reduction in duration of a symptom.

As used herein, "controlling post-traumatic headache" refers to maintaining or reducing severity or duration of one or more symptoms of post-traumatic headache or frequency of post-traumatic headache attacks in an individual (as compared to the level before treatment). For example, the duration or severity of head and/or neck pain, or frequency of attacks is reduced by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, or 70% in the individual as compared to the level before treatment.

As used herein, a "headache hour" refers to an hour during which a subject experiences headache. Headache hours can be expressed in terms of whole hours (e.g., one headache hour, two headache hours, three headache hours, etc.) or in terms of whole and partial hours (e.g., 0.5 headache hours, 1.2 headache hours, 2.67 headache hours, etc.). One or more headache hours may be described with respect to a particular time interval. For example, "daily headache hours" may refer to the number of headache hours a subject experiences within a day interval (e.g., a 24-hour period). In another example, "weekly headache hours" may refer to the number of headache hours a subject experiences within a week interval (e.g., a 7-day period). As can be appreciated, a week interval may or may not correspond to a calendar week. In another example, "monthly headache hours" may refer to the number of headache hours a subject experiences within a month interval. As can be appreciated, a month interval (e.g., a period of 28, 29, 30, or 31 days) may vary in terms of number of days depending

upon the particular month and may or may not correspond to a calendar month. In yet another example, "yearly headache hours" may refer to the number of headache hours a subject experiences within a year interval. As can be appreciated, a year interval (e.g., a period of 365 or 366 days) may vary in terms of number of days depending upon the particular year and may or may not correspond to a calendar year.

As used herein, a "headache day" refers to a day during which a subject experiences headache. Headache days can be expressed in terms of whole days (e.g., one headache day, two headache days, three headache days, etc.) or in terms of whole and partial days (e.g., 0.5 headache days, 1.2 headache days, 2.67 headache days, etc.). One or more headache days may be described with respect to a particular time interval. For example, "weekly headache days" may refer to the number of headache days a subject experiences within a week interval (e.g., a 7-day period). As can be appreciated, a week interval may or may not correspond to a calendar week. In another example, "monthly headache days" may refer to the number of headache days a subject experiences within a month interval. As can be appreciated, a month interval (e.g., a period of 28, 29, 30, or 31 days) may vary in terms of number of days depending upon the particular month and may or may not correspond to a calendar month. In yet another example, "yearly headache days" may refer to the number of headache days a subject experiences within a year interval. As can be appreciated, a year interval (e.g., a period of 365 or 366 days) may vary in terms of number of days depending upon the particular year and may or may not correspond to a calendar year.

As used therein, "delaying" the development of post-traumatic headache means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop post-traumatic headache. A method that "delays" development of the symptom is a method that reduces probability of developing the symptom in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not

using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of subjects.

"Development" or "progression" of post-traumatic headache means initial manifestations and/or ensuing progression of the disorder. Development of post-traumatic headache can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this disclosure, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of post-traumatic headache includes initial onset and/or recurrence.

As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the onset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing pain intensity, duration, or frequency of post-traumatic headache attack, and decreasing one or more symptoms resulting from post-traumatic headache (biochemical, histological and/or behavioral), including its complications and intermediate pathological phenotypes presenting during development of the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication, and/or delaying the progression of the disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this disclosure, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be

considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

An “individual” or a "subject" is a mammal, more preferably a human. Mammals also include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats.

As used herein, "vector" means a construct, which is capable of delivering, and preferably expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

As used herein, "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline or normal (0.9%) saline. Compositions comprising such carriers are formulated by well-known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA, 1990; and Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000).

The term "k_{on}", as used herein, is intended to refer to the rate constant for association of an antibody to an antigen.

The term "k_{off} ", as used herein, is intended to refer to the rate constant for dissociation of an antibody from the antibody/antigen complex.

The term "K_D", as used herein, is intended to refer to the equilibrium dissociation constant of an antibody-antigen interaction.

A. Methods for preventing, treating, or reducing post-traumatic headache and/or at least one secondary symptom associated with post-traumatic headache

In one aspect, the invention provides methods of preventing, treating, or reducing incidence of (persistent) post-traumatic headache in a subject. In another aspect, the invention provides a method of treating or reducing incidence of at least one secondary symptom associated with post-traumatic headache in a subject. In some embodiments, the method comprises administering to the individual an effective amount of an antibody or polypeptides derived from the antibody that modulates the CGRP pathway (e.g., a monoclonal anti-CGRP antagonist antibody).

In another aspect, the invention provides methods for preventing, ameliorating, controlling, reducing incidence of, or delaying the development or progression of (persistent) post-traumatic headache in an individual or symptoms associated with (persistent) post-traumatic headache (e.g., diarrhea, light sensitivity, fever, stiff neck, nausea, cognitive impairment and/or vomiting) comprising administering to the individual an effective amount of an antibody that modulates the CGRP pathway or an anti-CGRP antagonist antibody in combination with at least one additional agent useful for preventing, treating, or reducing (persistent) post-traumatic headache.

Such additional agents include, but are not limited to, 5-HT agonists and NSAIDs. For example, the antibody and the at least one additional agent can be concomitantly administered, i.e., they can be given in close enough temporal proximity to allow their individual therapeutic effects to overlap. For example, the amount of 5-HT agonist or NSAID administered in combination with an anti-CGRP antibody should be sufficient to reduce the frequency of (persistent) post-traumatic headache relapse in patients or produce longer lasting efficacy compared to the administration of either one of these agents in the absence of the other.

Additional non-limiting examples of additional agents that may be administered in combination with an anti-CGRP antagonist antibody include one or more of:

(i) an opioid analgesic, e.g., morphine, heroin, hydromorphone, oxymorphone, levorphanol, levallorphan, methadone, meperidine, fentanyl, cocaine, codeine, dihydrocodeine, oxycodone, hydrocodone, propoxyphene, nalmefene, nalorphine, naloxone, naltrexone, buprenorphine, butorphanol, nalbuphine or pentazocine;

(ii) a nonsteroidal antiinflammatory drug (NSAID), e.g., aspirin, diclofenac, diflusinal, etodolac, fenbufen, fenoprofen, flufenisal, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamic acid, mefenamic acid, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tolmetin or zomepirac, cyclooxygenase-2 (COX-2) inhibitors, celecoxib; rofecoxib; meloxicam; JTE-522; L-745,337; NS398; or a pharmaceutically acceptable salt thereof;

(iii) a barbiturate sedative, e.g., amobarbital, aprobarbital, butabarbital, butabital, mephobarbital, metharbital, methohexital, pentobarbital, phenobartital, secobarbital, talbutal, theamylal or thiopental or a pharmaceutically acceptable salt thereof;

(iv) a barbiturate analgesic, e.g., butalbital or a pharmaceutically acceptable salt thereof or a composition comprising butalbital.

(v) a benzodiazepine having a sedative action, e.g., chlordiazepoxide, clorazepate, diazepam, flurazepam, lorazepam, oxazepam, temazepam, or triazolam or a pharmaceutically acceptable salt thereof;

(vi) an H_1 antagonist having a sedative action, e.g., diphenhydramine, pyrilamine, promethazine, chlorpheniramine, or chlorcyclizine or a pharmaceutically acceptable salt thereof;

(vii) a sedative such as glutethimide, meprobamate, methaqualone or dichloralphenazone or a pharmaceutically acceptable salt thereof;

(viii) a skeletal muscle relaxant, e.g., baclofen, carisoprodol, chlorzoxazone, cyclobenzaprine, methocarbamol or orphrenadine or a pharmaceutically acceptable salt thereof;

(ix) an NMDA receptor antagonist, e.g., dextromethorphan ((+)-3-hydroxy-N-methylmorphinan) or its metabolite dextrorphan ((+)-3-hydroxy-N-methylmorphinan),

ketamine, memantine, pyrroloquinoline quinone or cis-4-(phosphonomethyl)-2-piperidinecarboxylic acid or a pharmaceutically acceptable salt thereof;

(x) an alpha-adrenergic, e.g., doxazosin, tamsulosin, clonidine or 4-amino-6,7-dimethoxy-2-(5-methanesulfonamido-1,2,3,4-tetrahydroisoquinol-2-yl)-5-(2-pyridyl) quinazoline;

(xi) a tricyclic antidepressant, e.g., desipramine, imipramine, amytriptiline or nortriptiline;

(xii) an anticonvulsant, e.g., carbamazepine or valproate;

(xiii) a tachykinin (NK) antagonist, particularly an NK-3, NK-2 or NK-1 antagonist, e.g., (αR,9R)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7H-[1,4]diazocino[2,1-g][1,7]naphthridine-6-13-dione (TAK-637), 5-[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one (MK-869), lanepitant, dapitant or 3-[[2-methoxy-5-(trifluoromethoxy)phenyl]methylamino]-2-phenyl-piperidine (2S,3S);

(xiv) a muscarinic antagonist, e.g., oxybutin, tolterodine, propiverine, tropsium chloride or darifenacin;

(xv) a COX-2 inhibitor, e.g., celecoxib, rofecoxib or valdecoxib;

(xvi) a non-selective COX inhibitor (preferably with GI protection), e.g., nitroflurbiprofen (HCT-1026);

(xvii) a coal-tar analgesic, in particular paracetamol;

(xviii) a neuroleptic such as droperidol;

(xix) a vanilloid receptor agonist (e.g., resinferatoxin) or antagonist (e.g., capsazepine);

(xx) a beta-adrenergic such as propranolol;

(xxi) a local anaesthetic, such as mexiletine;

(xxii) a corticosteroid, such as dexamethasone;

(xxiii) a serotonin receptor agonist or antagonist;

(xxiv) a cholinergic (nicotinic) analgesic;

(xxv) Tramadol (trade mark);

(xxvi) a PDEV inhibitor, such as sildenafil, vardenafil or taladafil;

(xxvii) an alpha-2-delta ligand such as gabapentin or pregabalin;

(xxviii) a canabinoid; and

(xxix) an antidepressant, such as amitriptyline (Elavil), trazodone (Desyrel), and imipramine (Tofranil) or anticonvulsants such as phenytoin (Dilantin) or carbamazepine (Tegretol).

Those skilled in the art will be able to determine appropriate dosage amounts for particular agents to be used in combination with an anti-CGRP antibody. For example, sumatriptan may be administered in a dosage from about 0.01 to about 300 mg. In some cases, sumatriptan may be administered in a dosage from about 2 mg to about 300 mg, e.g., about 5 mg to about 250 mg, about 5 mg to about 200 mg, about 5 mg to about 100 mg, about 5 mg to about 50 mg, or about 5 mg to about 25 mg. When administered non-parenterally, the typical dosage of sumatriptan is from about 25 to about 100 mg with about 50 mg being generally preferred, e.g., about 45 mg, about 55 mg, or about 60 mg. When sumatriptan is administered parenterally, the preferred dosage is about 6 mg, e.g., about 5 mg, about 7 mg, or about 8 mg. However, these dosages may be varied according to methods standard in the art so that they are optimized for a particular patient or for a particular combination therapy. Further, for example, celecoxib may be administered in an amount of between 50 and 500 mg, e.g., about 50 mg to about 400 mg, about 50 mg to about 300 mg, about 50 mg to about 200 mg, about 50 mg to about 100 mg, about 100 mg to about 400 mg, or about 200 mg to about 300 mg.

In another aspect, the disclosure provides a method of preventing, treating, or reducing incidence of (persistent) post-traumatic headache in a subject comprising administering to the subject a monoclonal antibody (e.g., a monoclonal, anti-CGRP antagonist antibody) that modulates the CGRP pathway. In some embodiments, the amount of the monoclonal antibody administered on each of the plurality of days may be between 0.1 mg – 5000 mg, 1 mg – 5000 mg, 10 mg – 5000 mg, 100 mg – 5000 mg, 1000 mg – 5000 mg, 0.1 mg - 4000 mg, 1 mg - 4000 mg, 10 mg – 4000 mg, 100 mg – 4000 mg, 1000 mg – 4000 mg, 0.1 mg - 3000 mg, 1 mg - 3000 mg, 10 mg - 3000 mg, 100 mg - 3000 mg, 1000 mg – 3000 mg, 0.1 mg - 2000 mg, 1 mg - 2000 mg, 10 mg – 2000 mg, 100 mg – 2000 mg, 1000 mg – 2000 mg, 0.1 mg – 1000 mg, 1 mg -1000 mg, 10 mg – 1000 mg or 100 mg – 1000 mg. In some embodiments, the amount is between

about 225 mg and about 1000 mg, e.g., about 675 mg or about 900 mg. An exemplary dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial antibody dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for one year, two years, three years, four years, or five years. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. In some embodiments, the initial dose and one or more of the additional doses are administered the same way, e.g., subcutaneously or intravenously. In some embodiments, the one or more additional doses are administered in a different way than the initial dose, e.g., the initial dose may be administered intravenously and the one or more additional doses may be administered subcutaneously.

In another aspect, the disclosure provides a method of preventing, treating, or reducing incidence of (persistent) post-traumatic headache in a subject comprising administering to the subject a single dose of a monoclonal antibody (e.g., a monoclonal, anti-CGRP antagonist antibody) in an amount that modulates the CGRP pathway. In some embodiments, the single dose may be an amount of antibody between 0.1 mg - 5000 mg, 1 mg - 5000 mg, 10 mg -5000 mg, 100 mg - 5000 mg, 1000 mg – 5000 mg, 0.1 mg - 4000 mg, 1 mg - 4000 mg, 10 mg - 4000 mg, 100 mg - 4000 mg, 1000 mg – 4000 mg, 0.1 mg - 3000 mg, 1 mg - 3000 mg, 10 mg - 3000 mg, 100 mg - 3000 mg, 1000 mg – 3000 mg, 0.1 mg - 2000 mg, 1 mg - 2000 mg, 10 mg - 2000 mg, 100 mg - 2000 mg, 1000 mg – 2000 mg, 0.1 mg - 1000 mg, 1 mg -1000 mg, 10 mg - 1000 mg or 100 mg - 1000 mg. In some embodiments, the single dose may be an amount of antibody between 225 mg and about 1000 mg, e.g., about 675 mg or about 900 mg.

In another aspect, the disclosure provides a method of preventing, treating, or reducing incidence of (persistent) post-traumatic headache in a subject comprising administering to the subject a monthly dose of a monoclonal antibody (e.g., a

monoclonal, anti-CGRP antagonist antibody) in an amount that modulates the CGRP pathway. In some embodiments, the single dose may be an amount of antibody between 0.1 mg - 5000 mg, 1 mg - 5000 mg, 10 mg -5000 mg, 100 mg - 5000 mg, 1000 mg – 5000 mg, 0.1 mg - 4000 mg, 1 mg - 4000 mg, 10 mg - 4000 mg, 100 mg - 4000 mg, 1000 mg – 4000 mg, 0.1 mg - 3000 mg, 1 mg - 3000 mg, 10 mg - 3000 mg, 100 mg - 3000 mg, 1000 mg – 3000 mg, 0.1 mg - 2000 mg, 1 mg - 2000 mg, 10 mg - 2000 mg, 100 mg - 2000 mg, 1000 mg – 2000 mg, 0.1 mg - 1000 mg, 1 mg -1000 mg, 10 mg - 1000 mg or 100 mg - 1000 mg. In some embodiments, the monthly dose may be an amount of antibody between about 225 mg and about 1000 mg, e.g., about 675 mg or about 900 mg. An exemplary dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial antibody dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for one year, two years, three years, four years, or five years. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. In some embodiments, the initial dose and one or more of the additional doses are administered the same way, e.g., subcutaneously or intravenously. In some embodiments, the one or more additional doses are administered in a different way than the initial dose, e.g., the initial dose may be administered intravenously and the one or more additional doses may be administered subcutaneously.

In another aspect, the disclosure provides a method of decreasing a number of monthly headache hours experienced by a subject, comprising administering to the subject an amount of a monoclonal antibody (e.g., a monoclonal, anti-CGRP antagonist antibody) that modulates the CGRP pathway. In some embodiments, the monoclonal antibody can be in an amount effective to decrease the number of monthly headache hours by at least 0.1, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100 or more headache hours after a single dose, monthly dose, or quarterly

dose. In some embodiments, the monoclonal antibody can be in an amount effective to decrease the number of monthly headache hours by at least 20 headache hours after a single dose, monthly dose, or quarterly dose. In some embodiments, the monoclonal antibody can be in an amount effective to decrease the number of monthly headache hours by at least 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or more headache hours. In some embodiments, the monoclonal antibody can be in an amount effective to decrease the number of monthly headache hours by at least 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more after a single dose. In some embodiments, the monoclonal can be in an amount effective to decrease the number of monthly headache hours by at least 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more after a single dose, monthly dose, or quarterly dose.

In another aspect, the disclosure provides a method of decreasing a number of monthly headache days experienced by a subject, comprising administering to the subject an amount of a monoclonal antibody (e.g., a monoclonal, anti-CGRP antagonist antibody) that modulates the CGRP pathway. In some embodiments, the monoclonal antibody can be in an amount effective to decrease the number of monthly headache days by at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more headache days after a single dose. In some embodiments, the monoclonal antibody can be in an amount effective to decrease the number of monthly headache days by at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more headache days after a monthly dose or quarterly dose. In some embodiments, the monoclonal antibody can be in an amount effective to decrease the number of monthly headache days by at least 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more after a single dose, monthly dose, or quarterly dose.

In another aspect, the disclosure provides a method of decreasing use of an anti-headache medication in a subject, comprising administering to the subject a monoclonal antibody (e.g., a monoclonal anti-CGRP antagonist antibody) that

modulates the CGRP pathway. In some embodiments, the monoclonal antibody can be in an amount effective to decrease daily, monthly, quarterly, and/or yearly use of the anti-headache medication by the subject by at least 0.1%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more. In some embodiments, the monoclonal antibody can be in an amount effective to decrease monthly use of the anti-headache medication by the subject by at least 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more. The anti-headache medication can be any type of anti-headache medication described herein. Non-limiting examples of anti-headache medications include, for example, 5-HT1 agonists (and agonists acting at other 5-HT1 sites), triptans (e.g., sumatriptan, zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan, afrovatriptan), ergot alkaloids (e.g., ergotamine tartrate, ergonovine maleate, and ergoloid mesylates (e.g., dihydroergocornine, dihydroergocristine, dihydroergocryptine, and dihydroergotamine mesylate (DHE 45)) and non-steroidal anti-inflammatory drugs (NSAIDs) (e.g., aspirin, diclofenac, diflusinal, etodolac, fenbufen, fenoprofen, flufenisal, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamic acid, mefenamic acid, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac, tolmetin or zomepirac, cyclooxygenase-2 (COX-2) inhibitors, celecoxib; rofecoxib; meloxicam; JTE-522; L-745,337; NS398; or a pharmaceutically acceptable salt thereof), opiates (e.g., oxycodone), and β-adrenergic antagonists (e.g., propranolol).

With respect to all methods described herein, references to antibodies (e.g., monoclonal antibodies that modulate the CGRP pathway, anti-CGRP antagonist antibodies, monoclonal anti-CGRP antagonist antibodies) also include compositions comprising one or more of these agents. Accordingly, such a composition may be used according to a method referring to an antibody described herein. These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients as described elsewhere herein. The present invention can be used alone or in combination with other conventional methods of treatment.

An antibody described herein (e.g., a monoclonal antibody, an anti-CGRP antagonist antibody, a monoclonal anti-CGRP antagonist antibody) can be administered to an individual or subject in any therapeutic dose, via any suitable route and in any suitable formulation. It should be apparent to a person skilled in the art that the examples described herein are not intended to be limiting but to be illustrative of the techniques available. Accordingly, in some embodiments, an antibody described herein can be administered to a subject in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, e.g., about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 60 minutes, about 90 minutes, about 120 minutes, about 180 minutes, or about 240 minutes. The antibody described herein can also be administered to the subject by subcutaneous, intramuscular, intraperitoneal, intracerebrospinal, intra-articular, sublingually, intra-arterial, intrasynovial, via insufflation, intrathecal, oral, inhalation, intranasal (e.g., with or without inhalation), buccal, rectal, transdermal, intracardiac, intraosseous, intradermal, transmucosal, vaginal, intravitreal, peri-articular, local, epicutaneous, or topical routes. Administration can be systemic, e.g., intravenous administration, or localized. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, an antibody described herein can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

In some embodiments, an antibody described herein can be administered via site-specific or targeted local delivery techniques. Examples of site-specific or targeted local delivery techniques include various implantable depot sources of the antibody or local delivery catheters, such as infusion catheters, an indwelling catheter, or a needle catheter, synthetic grafts, adventitial wraps, shunts and stents or other implantable devices, site specific carriers, direct injection, or direct application. See e.g., PCT Publication No. WO 00/53211 and U.S. Patent No. 5,981,568, which are hereby incorporated by reference in their entireties.

Various formulations of an antibody described herein may be used for administration. In some embodiments, an antibody may be administered neat. In some embodiments, antibody and a pharmaceutically acceptable excipient may be in various formulations. Pharmaceutically acceptable excipients are known in the art, and are relatively inert substances that facilitate administration of a pharmacologically effective substance. For example, an excipient can give form or consistency, or act as a diluent. Suitable excipients include but are not limited to stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers. Excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

In some embodiments, these agents, including antibodies described herein, may be formulated for administration by injection (e.g., intravenously, subcutaneously, intraperitoneally, intramuscularly, etc.). Accordingly, these agents can be combined with pharmaceutically acceptable vehicles such as saline, Ringer's solution, dextrose solution, and the like. The particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular individual and that individual's medical history.

In some embodiments, these agents, including antibodies described herein, may be formulated for peripheral administration. Such formulations can be administered peripherally via any suitable peripheral route, including intravenously and subcutaneously. An agent prepared for peripheral administration can include a substance, medicament, and/or antibody that is not delivered centrally, spinally, intrathecally, or directly into the CNS. Non-limiting examples of peripheral administration routes include a route which is oral, sublingual, buccal, topical, rectal, via inhalation, transdermal, subcutaneous, intravenous, intra-arterial, intramuscular, intracardiac, intraosseous, intradermal, intraperitoneal, transmucosal, vaginal, intravitreal, intra-articular, peri-articular, local, or epicutaneous.

Therapeutic formulations of the antibodies used in accordance with the present disclosure can be prepared for storage and/or use by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients

or stabilizers (Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000)), and can in some cases be in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed. A therapeutic formulation of an antibody may comprise one or more pharmaceutically acceptable carriers, excipients or stabilizes with non-limiting examples of such species that include buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids (e.g., at concentrations of 0.1 mM to 100 mM, 0.1 mM to 1 mM, 0.01 mM to 50 mM, 1 mM to 50 mM, 1 mM to 30 mM, 1 mM to 20 mM, 10 mM to 25 mM) such as glycine, glutamine, methionine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents (e.g., at concentrations of 0.001 mg/mL to 1 mg/mL, 0.001 mg/mL to 1 mg/mL, 0.001 mg/mL to 0.1 mg/mL, 0.001 mg/mL to 0.01 mg/mL, 0.01 mg/mL to 0.1 mg/mL) such as EDTA (e.g., disodium EDTA dihydrate); sugars (e.g., at concentrations of 1 mg/mL to 500 mg/mL, 10 mg/mL to 200 mg/mL, 10 mg/mL to 100 mg/mL, 50 mg/mL to 150 mg/mL) such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants (e.g., at concentrations of 0.01 mg/mL to 10 mg/mL, 0.01 mg/mL to 1 mg/mL, 0.1 mg/mL to 1 mg/mL, 0.01 mg/mL to 0.5 mg/mL) such as TWEEN™ (e.g., polysorbate (e.g., polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80)), PLURONICS™ or polyethylene glycol (PEG).

An antibody formulation may be characterized in terms of any of a variety of physical properties. For example, a liquid antibody formulation may have any suitable pH for therapeutic efficacy, safety and storage. For example, the pH of a liquid antibody

formulation may be from pH 4 to about pH 9, from about pH 5 to about pH 8, from about pH 5 to about pH 7 or from about pH 6 to about pH 8. In some embodiments, a liquid antibody formulation may have a pH of about 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, or about 10 or higher or lower.

In another example, a liquid antibody formulation may have any suitable viscosity for therapeutic efficacy, safety and storage. For example, the viscosity of a liquid antibody formulation may be from about 0.5 centipoise (cP) to about 100 cP, about 1 cP to about 50 cP, about 1 cP to about 20 cP, about 1 cP to about 15 cP, or about 5 cP to about 15 cP at 25ºC. In some embodiments, a liquid antibody formulation may have a viscosity of about 0.5 cP, 1 cP, 1.2 cP, 1.4 cP, 1.6 cP, 1.8 cP, 2.0 cP, 2.2 cP, 2.4 cP, 2.6 cP, 2.8 cP, 3.0 cP, 3.2 cP, 3.4 cP, 3.6 cP, 3.8 cP, 4.0 cP, 4.2 cP, 4.4 cP, 4.6 cP, 4.8 cP, 5.0 cP, 5.2 cP, 5.4 cP, 5.6 cP, 5.8 cP, 6.0 cP, 6.2 cP, 6.4 cP, 6.6 cP, 6.8 cP, 7.0 cP, 7.2 cP, 7.4 cP, 7.6 cP, 7.8 cP, 8.0 cP, 8.2 cP, 8.4 cP, 8.6 cP, 8.8 cP, 9.0 cP, 9.2 cP, 9.4 cP, 9.6 cP, 9.8 cP, 10.0 cP, 10.2 cP, 10.4 cP, 10.6 cP, 10.8 cP, 11.0 cP, 11.2 cP, 11.4 cP, 11.6 cP, 11.8 cP, 12.0 cP, 12.2 cP, 12.4 cP, 12.6 cP, 12.8 cP, 13.0 cP, 13.2 cP, 13.4 cP, 13.6 cP, 13.8 cP, 14.0 cP, 14.2 cP, 14.4 cP, 14.6 cP, 14.8 cP, or about 15.0 cP at 25ºC or the viscosity may be higher or lower.

In another example, a liquid antibody formulation may have any suitable conductivity for therapeutic efficacy, safety and storage. For example, the conductivity of a liquid antibody formulation may be from about 0.1 millisiemens per centimeter (mS/cm) to about 15 mS/cm, 0.1 mS/cm to 10 mS/cm, 0.1 mS/cm to 5 mS/cm, 0.1 mS/cm to 2 mS/cm or 0.1 mS/cm to 1.5 mS/cm. In some embodiments, a liquid antibody formulation may have a conductivity of 0.19 mS/cm, 0.59 mS/cm, 1.09 mS/cm, 1.19 mS/cm, 1.29 mS/cm, 1.39 mS/cm, 1.49 mS/cm, 1.59 mS/cm, 1.69 mS/cm, 1.79 mS/cm, 1.89 mS/cm, 1.99 mS/cm, 2.09 mS/cm, 2.19 mS/cm, 2.29 mS/cm, 2.39 mS/cm, 2.49 mS/cm, 2.59 mS/cm, 2.69 mS/cm, 2.79 mS/cm, 2.89 mS/cm, 2.99 mS/cm, 3.09 mS/cm, 3.19 mS/cm, 3.29 mS/cm, 3.39 mS/cm, 3.49 mS/cm, 3.59 mS/cm, 3.69 mS/cm, 3.79 mS/cm, 3.89 mS/cm, 3.99 mS/cm, 4.09 mS/cm, 4.19 mS/cm, 4.29 mS/cm, 4.39 mS/cm, 4.49 mS/cm, 4.59 mS/cm, 4.69 mS/cm, 4.79 mS/cm, 4.89 mS/cm, 4.99 mS/cm, 5.09 mS/cm, 6.09 mS/cm, 6.59 mS/cm, 7.09 mS/cm, 7.59 mS/cm, 8.09 mS/cm, 8.59

mS/cm, 9.09 mS/cm, 9.59 mS/cm, 10.09 mS/cm, 10.59 mS/cm, 11.09 mS/cm, 11.59 mS/cm, 12.09 mS/cm, 12.59 mS/cm, 13.09 mS/cm, 13.59 mS/cm, 14.09 mS/cm, 14.59 mS/cm, or about 15.09 mS/cm or the conductivity may be higher or lower.

In another example, a liquid antibody formulation may have any suitable osmolality for therapeutic efficacy, safety, and storage. For example, the osmolality of a liquid antibody formulation may be from about 50 milliosmole per kilogram (mOsm/kg) to about 5000 mOsm/kg, about 50 mOsm/kg to about 2000 mOsm/kg, about 50 mOsm/kg to about 1000 mOsm/kg, about 50 mOsm/kg to about 750 mOsm/kg, or about 50 mOsm/kg to about 500 mOsm/kg. In some embodiments, a liquid antibody formulation may have an osmolality of about 50 mOsm/kg, 60 mOsm/kg, 70 mOsm/kg, 80 mOsm/kg, 90 mOsm/kg, 100 mOsm/kg 120 mOsm/kg, 140 mOsm/kg, 160 mOsm/kg, 180 mOsm/kg, 200 mOsm/kg, 220 mOsm/kg, 240 mOsm/kg, 260 mOsm/kg, 280 mOsm/kg, 300 mOsm/kg, 320 mOsm/kg, 340 mOsm/kg, 360 mOsm/kg, 380 mOsm/kg, 400 mOsm/kg, 420 mOsm/kg, 440 mOsm/kg, 460 mOsm/kg, 480 mOsm/kg, 500 mOsm/kg, 520 mOsm/kg, 540 mOsm/kg, 560 mOsm/kg, 580 mOsm/kg, 600 mOsm/kg, 620 mOsm/kg, 640 mOsm/kg, 660 mOsm/kg, 680 mOsm/kg, 700 mOsm/kg, 720 mOsm/kg, 740 mOsm/kg, 760 mOsm/kg, 780 mOsm/kg, 800 mOsm/kg, 820 mOsm/kg, 840 mOsm/kg, 860 mOsm/kg, 880 mOsm/kg, 900 mOsm/kg, 920 mOsm/kg, 940 mOsm/kg, 960 mOsm/kg, 980 mOsm/kg, 1000 mOsm/kg, 1050 mOsm/kg, 1100 mOsm/kg, 1150 mOsm/kg, 1200 mOsm/kg, 1250 mOsm/kg, 1300 mOsm/kg, 1350 mOsm/kg, 1400 mOsm/kg, 1450 mOsm/kg, about 1500 mOsm/kg, or the osmolality may be higher or lower.

Liposomes containing antibody can be prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl Acad. Sci. USA 77:4030 (1980); and U.S. Patent Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or 'poly(v nylalcohol)), polylactides (U.S. Patent No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

The formulations to be used for in vivo administration should generally be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. Therapeutic antibody compositions are generally placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The compositions according to the present invention may be in unit dosage forms such as tablets, pills, capsules, powders, granules, solutions or suspensions, or suppositories, for oral, parenteral or rectal administration, or administration by inhalation or insufflation. In some cases, a unit dosage form may be supplied in a prefilled receptacle (e.g., a prefilled syringe) useful in administering the unit dosage to a subject.

For preparing solid compositions such as tablets, the principal active ingredient can be mixed with a pharmaceutical carrier, e.g., conventional tableting ingredients such as corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate,

dicalcium phosphate, or gums, and other pharmaceutical diluents, e.g., water, to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention, or a non-toxic pharmaceutically acceptable salt thereof. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules. This solid preformulation composition is then subdivided into unit dosage forms of the type described above containing from about 0.1 mg to about 500 mg of the active ingredient of the present invention. The tablets or pills of the novel composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permits the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

Suitable surface-active agents include, in particular, non-ionic agents, such as polyoxyethylenesorbitans (e.g., TWEEN™ 20, 40, 60, 80, or 85) and other sorbitans (e.g., SPAN™ 20, 40, 60, 80, or 85). Compositions with a surface-active agent will conveniently comprise between about 0.05 and about 5% surface-active agent, and can be between about 0.1% and about 2.5%. It will be appreciated that other ingredients may be added, for example mannitol or other pharmaceutically acceptable vehicles, if necessary.

Suitable emulsions may be prepared using commercially available fat emulsions, such as INTRALIPID™, LIPOSYN™, INFONUTROL™, LIPOFUNDIN™, and LIPIPHYSAN™. The active ingredient may be either dissolved in a pre-mixed emulsion composition or alternatively it may be dissolved in an oil (e.g., soybean oil, safflower oil, cottonseed oil, sesame oil, corn oil or almond oil) and an emulsion formed upon mixing

with a phospholipid (e.g., egg phospholipids, soybean phospholipids, or soybean lecithin) and water. It will be appreciated that other ingredients may be added, for example glycerol or glucose, to adjust the tonicity of the emulsion. Suitable emulsions will typically contain up to 20% oil, for example, between 5 and 20%. The fat emulsion can comprise fat droplets between about 0.1 and 1.0 1m, particularly about 0.1 and 0.5 1m, and have a pH in the range of about pH 5.5 to about pH 8.0.

The emulsion compositions can be those prepared by mixing an antibody with INTRALIPID™ or the components thereof (soybean oil, egg phospholipids, glycerol and water).

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as set out above. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably sterile pharmaceutically acceptable solvents may be nebulised by use of gases. Nebulised solutions may be breathed directly from the nebulising device or the nebulising device may be attached to a face mask, tent or intermittent positive pressure breathing machine. Solution, suspension or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

In some embodiments, a formulation comprising an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein may be prepared for any suitable route of administration with an antibody amount ranging from about 0.1 mg to about 3000 mg, about 1 mg to about 1000 mg, about 100 mg to about 1000 mg, or about 100 mg to about 500 mg. In some cases, a formulation comprising an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein may comprise an antibody amount of, at most, or at least about 0.1 mg, 1 mg, 100 mg, 1 mg, 10 mg, 25 mg, 50 mg, 75 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 225 mg, 250 mg, 275

mg, 300 mg, 325 mg, 350 mg, 375 mg, 400 mg, 450 mg, 475 mg, 500 mg, 525 mg, 550 mg, 575 mg, 600 mg, 625 mg, 650 mg, 675 mg, 700 mg, 725 mg, 750 mg, 775 mg, 800 mg, 825 mg, 850 mg, 875 mg, 900 mg, 925 mg, 950 mg, 975 mg, 1000 mg, 1100 mg, 1200 mg, 1300 mg, 1400 mg, 1500 mg, 1600 mg, 1700 mg, 1800 mg, 1900 mg, 2000 mg, or about 3000 mg.

In some embodiments, a liquid formulation comprising an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein may be prepared for any suitable route of administration with an antibody concentration ranging from about 0.1 mg/mL to about 500 mg/mL, about 0.1 mg/mL to about 375 mg/mL, about 0.1 mg/mL to about 250 mg/mL, about 0.1 to about 175 mg/mL, about 0.1 to 100 mg/mL, about 1 mg/mL to about 500 mg/mL, about 1 mg/mL to about 375 mg/mL, about 1 mg/mL to about 300 mg/mL, about 1 mg/mL to 250 mg/mL, about 1 mg/mL to 200 mg/mL, about 1 mg/mL to 150 mg/mL, about 1 mg/mL to about 100 mg/mL, about 10 mg/ mL to 500 mg/mL, about 10 mg/mL to about 375 mg/mL, about 10 mg/mL to 250 mg/mL, about 10 mg/mL to about 150 mg/mL, about 10 mg/mL to 100 mg/mL, about 100 mg/mL to 500 mg/mL, about 100 mg/mL to 450 mg/mL, about 100 mg/mL to 400 mg/mL, about 100 mg/mL to about 350 mg/mL, about 100 mg/mL to about 300 mg/mL, about 100 mg/mL to about 250 mg/mL, 100 mg/mL to 200 mg/mL, or about 100 mg/mL to about 150 mg/mL. In some embodiments, a liquid formulation may comprise an antibody described herein at a concentration of, of at most, of at least, or less than about 0.1, 0.5, 1, 5, 10,15 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105 110, 115, 120, 125, 130, 135, 140, 145, 150, 155, 160, 165, 170, 175, 180, 185, 190, 195, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, or about 500 mg/mL.

An antibody formulation may comprise one or more components including the antibody and other species described elsewhere herein. The antibody and other components may be in any suitable amount and/or any suitable concentration for therapeutic efficacy of the antibody, safety and storage. In one example, an antibody

formulation may be a solution comprising about 51.4 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 16-20 mM histidine, 0.1 mg/mL methionine, 84 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, and 0.2 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 200 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 15 mM arginine, 78 mg/mL sucrose, 0.3 mg/mL EDTA, and 0.1 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 175 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 20 mM glycine, 88 mg/mL trehalose dihydrate, 0.015 mg/mL EDTA, and 0.25 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 225 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 23 mM asparagine, 84 mg/mL sorbitol, 0.1 mg/mL EDTA, and 0.15 mg/mL polysorbate 60.

In another example, an antibody formulation may comprise about 150 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 17 mM asparagine, 74 mg/mL mannitol, 0.025 mg/mL EDTA, and 0.2 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 100 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 16 mM arginine, 87 mg/mL mannitol, 0.025 mg/mL EDTA, and 0.15 mg/mL polysorbate 20.

In another example, an antibody formulation may comprise about 250 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 25 mM histidine, 74 mg/mL mannitol, 0.025 mg/mL EDTA, and 0.25 mg/mL polysorbate 20.

In another example, an antibody formulation may comprise about 50 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal

antibody that modulates the CGRP pathway), 19 mM arginine, 84 mg/mL sucrose, 0.05 mg/mL EDTA, and 0.3 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 125 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 22 mM glycine, 79 mg/mL trehalose dihydrate, 0.15 mg/mL EDTA, and 0.15 mg/mL polysorbate 80.

In another example, an antibody formulation may be a solution comprising about 175 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 20 mM histidine, 0.1 mg/mL methionine, 84 mg/mL trehalose dihydrate, 0.05 mg/mL disodium EDTA dihydrate, and 0.2 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 200 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 30 mM arginine, 78 mg/mL sucrose, 0.3 mg/mL EDTA, and 0.1 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 175 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 20 mM glycine, 88 mg/mL trehalose dihydrate, 0.015 mg/mL EDTA, and 0.15 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 150 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 20 mM histidine, 84 mg/mL sucrose, 0.05 mg/mL EDTA, and 0.2 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 225 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 23 mM histidine, 84 mg/mL sorbitol, 0.1 mg/mL EDTA, and 0.15 mg/mL polysorbate 60.

In another example, an antibody formulation may comprise about 150 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal

antibody that modulates the CGRP pathway), 17 mM asparagine, 74 mg/mL mannitol, 0.3 mg/mL EDTA, and 0.2 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise about 100 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 16 mM arginine, 87 mg/mL mannitol, 0.025 mg/mL EDTA, and 0.25 mg/mL polysorbate 20.

In another example, an antibody formulation may comprise about 250 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 25 mM histidine, 89 mg/mL mannitol, 0.025 mg/mL EDTA, and 0.25 mg/mL polysorbate 20.

In another example, an antibody formulation may comprise 125 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 29 mM arginine, 84 mg/mL sucrose, 0.05 mg/mL EDTA, and 0.3 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise 150 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 25 mM asparagine, 84 mg/mL mannitol, 0.05 mg/mL EDTA, and 0.2 mg/mL polysorbate 80.

In another example, an antibody formulation may comprise 145 mg/mL antibody (e.g., antibody G1, another anti-CGRP antagonist antibody, or a monoclonal antibody that modulates the CGRP pathway), 22 mM histidine, 72 mg/mL trehalose dihydrate, 0.05 mg/mL EDTA, and 0.1 mg/mL polysorbate 80.

An antibody described herein can be administered using any suitable method, including by injection (e.g., intravenously, subcutaneously, intraperitoneally, intramuscularly, etc.). Antibodies can also be administered via inhalation, as described herein. In some cases, an antibody may be administered nasally with or without inhalation. Generally, for administration of an antibody described herein, an initial candidate dosage can be about 2 mg/kg. For the purpose of the present invention, a typical daily dosage might range from about any of 3 µg/kg to 30 µg/kg to 300 µg/kg to 3 mg/kg, to 30 mg/kg to 100 mg/kg or more, depending on the factors mentioned above.

For example, dosage of about 1 mg/kg, about 2.5 mg/kg, about 5 mg/kg, about 10 mg/kg, about 25 mg/kg, and about 30 mg/kg may be used. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved, for example, to reduce pain. An exemplary dosing regimen comprises administering an initial dose of about 8.5 mg/kg, or about 10 mg/kg, followed by a maintenance dose of about 2.8 mg/kg of an antibody, or followed by a maintenance dose of about 2.8 mg/kg every other week. Another exemplary dosing regimen comprises administering a dose of about 100 mg, 125 mg, 150 mg, 200 mg, 225 mg, 250 mg, 275 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, about 675 mg, or about 900 mg to a subject once per month intravenously in an infusion over about one hour, or subcutaneously. Another exemplary dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for one year, two years, three years, four years, or five years. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. For example, in some embodiments, dosing from about one to about four times a week is contemplated. The progress of this therapy is easily monitored by conventional techniques and assays. The dosing regimen (including the CGRP antagonist(s) used) can vary over time.

In some embodiments, the dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein and administered to a subject may range from about 0.1 μg to about 3000 mg, 1 mg to 1000 mg, 100 mg to 1000 mg, 100 mg to 500 mg, 0.1 mg to 5000 mg, 1 mg to 4000 mg, 250 mg to 1000 mg, 500 mg to 1000 mg, 100 mg to 900 mg, 400 mg to 900 mg, 10 mg to 3000 mg, 10 mg to

2000 mg, 100 mg to 2000 mg, 150 mg to 2000 mg, 200 mg to 2000 mg, 250 mg to 2000 mg, 300 mg to 2000 mg, 350 mg to 2000 mg, 400 mg to 2000 mg, 450 mg to 2000 mg, 500 mg to 2000 mg, 550 mg to 2000 mg, 600 mg to 2000 mg, 650 mg to 2000 mg, 700 mg to 2000 mg, 750 mg to 2000 mg, 800 mg to 2000 mg, 850 mg to 2000 mg, 900 mg to 2000 mg, 950 mg to 2000 mg, or 1000 mg to 2000 mg. In some embodiments, the dose or amount of an antibody described herein and administered to a subject may be, may be at most, may be less than, or may be at least about 0.1 μg, 1 μg, 100 μg, 1 mg, 10 mg, 25 mg, 50 mg, 75 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 225 mg, 250 mg, 275 mg, 300 mg, 325 mg, 350 mg, 375 mg, 400 mg, 450 mg, 475 mg, 500 mg, 525 mg, 550 mg, 575 mg, 600 mg, 625 mg, 650 mg, 675 mg, 700 mg, 725 mg, 750 mg, 775 mg, 800 mg, 825 mg, 850 mg, 875 mg, 900 mg, 925 mg, 950 mg, 975 mg, 1000 mg, 1100 mg, 1200 mg, 1300 mg, 1400 mg, 1500 mg, 1600 mg, 1700 mg, 1800 mg, 1900 mg, 2000 mg, or about 3000 mg. In some embodiments, the amount is between about 225 mg to about 1000 mg, e.g., about 675 mg or about 900 mg. An exemplary dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for one year, two years, three years, four years, or five years. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve.

In some embodiments, the dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein and administered to a subject may range from about 0.1 to 500, 0.1 to 100, 0.1 to 50, 0.1 to 20, 0.1 to 10, 1 to 10, 1 to 7, 1 to 5 or 0.1 to 3 mg/kg of body weight. In some embodiments, the dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described

herein and administered to a subject may be, may be at most, may be less than, or may be at least about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0, 15.5, 16.0, 16.5, 17.0, 17.5, 18.0, 18.5, 19.0, 19.5, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or about 500 mg/kg of body weight.

In some embodiments, the frequency at which a dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein is administered to a subject may vary. In some embodiments, a single dose of antibody may be given to a subject across therapy. In some embodiments, the frequency at which a dose or amount of an antibody is administered to a subject is constant (e.g., administered about once per month or about once per quarter). In some embodiments, the frequency at which a dose or amount of an antibody is administered to a subject is about every quarter for about one year, two years, three years, four years, or five years. In some embodiments, the frequency at which a dose or amount of an antibody described herein is administered to a subject is variable (e.g., an initial dose followed by a dose at once per month, followed by additional doses at about three months and about seven months). In some embodiments, the frequency at which an antibody is administered to a subject is, is at least, is less than, or is at most about one, two, three, four, five, or six time(s) per day. In some embodiments, the frequency at which an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) is administered to a subject is, is at least, is less than, or is at most about one, two, three, four, five, or six dose(s) per day.

In some embodiments, the frequency at which a dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein is administered

to a subject is, is at least, is less than, or is at most about one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty time(s) per every one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-five, sixty, sixty-five, seventy, seventy-five, eighty, eighty-five, ninety, ninety-five, one-hundred, one-hundred twenty-five, one-hundred fifty, one-hundred eighty, or two-hundred day(s).

In some embodiments, the frequency at which a dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein is administered to a subject is, is at least, is less than, or is at most about one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty time(s) per every one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty, thirty-one, thirty-two, thirty-three, thirty-four, thirty-five, thirty-six, thirty-seven, thirty-eight, thirty-nine, forty, forty-one, forty-two, forty-three, forty-four, forty-five, forty-six, forty-seven, forty-eight, forty-nine, fifty, fifty-five, sixty, sixty-five, seventy, seventy-five, eighty, eighty-five, ninety, ninety-five, or one-hundred week(s). In some embodiments, the frequency at which an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein is administered to a subject is less than or about one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen dose(s) per week.

In some embodiments, the frequency at which a dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist

antibody, monoclonal anti-CGRP antagonist antibody) is administered to a subject is, is at least, is less than, or is at most about one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty time(s) per every month, every two months, every three months, every four months, every five months, every six months, every seven months, every eight months, every nine months, every ten months, every eleven months, every twelve months, every thirteen months, every fourteen months, every fifteen months, every sixteen months, every seventeen months, or every eighteen month(s). In some embodiments, the frequency at which a dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) is administered to a subject is about one time per every one month. In some embodiments, the frequency at which a dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) is administered to a subject is about one time per every three months. In some embodiments, the frequency at which an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein is administered to a subject is less than about one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or fifteen dose(s) per month. In some embodiments, a dose or amount of an antibody may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject one time, two times, three times, four times, five times, six times, seven times, eight times, nine times, ten times or more per month.

In some embodiments, an antibody in a dose or amount of about 50 mg, 100 mg 150 mg, 200 mg, 225 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 675 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1000 mg, 1050 mg, 1100 mg, 1150 mg, 1200 mg, 1250 mg, 1300 mg, 1350 mg, 1400 mg, 1450 mg, 1500 mg, 1550 mg, 1600 mg, 1650 mg, 1700 mg, 1750 mg, 1800 mg, 1850 mg, 1900 mg, 1950 mg, 2000 mg, 2050 mg, 2100 mg, 2150 mg, 2200 mg, 2250 mg, 2300 mg, 2350 mg, 2400 mg, 2450 mg, 2500 mg, 2550 mg, 2600 mg, 2650 mg, 2700

mg, 2750 mg, 2800 mg, 2850 mg, 2900 mg, 2950 mg, 3000 mg, or more may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject once per month. In some embodiments, an antibody in a dose or amount of between about 0.1 mg to 5000 mg, 1 mg to 4000 mg, 10 mg to 3000 mg, 10 mg to 2000 mg, 100 mg to 2000 mg, 150 mg to 2000 mg, 200 mg to 2000 mg, 250 mg to 2000 mg, 300 mg to 2000 mg, 350 mg to 2000 mg, 400 mg to 2000 mg, 450 mg to 2000 mg, 500 mg to 2000 mg, 550 mg to 2000 mg, 600 mg to 2000 mg, 650 mg to 2000 mg, 700 mg to 2000 mg, 750 mg to 2000 mg, 800 mg to 2000 mg, 850 mg to 2000 mg, 900 mg to 2000 mg, 950 mg to 2000 mg, or about 1000 mg to 2000 mg may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject once per month. In some embodiments, between about 225 mg and about 1000 mg, e.g., about 225 mg of antibody are administered once per month. An exemplary dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for one year, two years, three years, four years, or five years. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve.

In some embodiments, an antibody in a dose or amount of about 50 mg, 100 mg 150 mg, 200 mg, 225 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 675 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1000 mg, 1050 mg, 1100 mg, 1150 mg, 1200 mg, 1250 mg, 1300 mg, 1350 mg, 1400 mg, 1450 mg, 1500 mg, 1550 mg, 1600 mg, 1650 mg, 1700 mg, 1750 mg, 1800 mg, 1850 mg, 1900 mg, 1950 mg, 2000 mg, 2050 mg, 2100 mg, 2150 mg, 2200 mg, 2250 mg, 2300 mg, 2350 mg, 2400 mg, 2450 mg, 2500 mg, 2550 mg, 2600 mg, 2650 mg, 2700 mg, 2750 mg, 2800 mg, 2850 mg, 2900 mg, 2950 mg, 3000 mg, or more may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject every

three months. In some embodiments, an antibody in a dose or amount of between about 0.1 mg to 5000 mg, 1 mg to 4000 mg, 10 mg to 3000 mg, 10 mg to 2000 mg, 100 mg to 2000 mg, 150 mg to 2000 mg, 200 mg to 2000 mg, 250 mg to 2000 mg, 300 mg to 2000 mg, 350 mg to 2000 mg, 400 mg to 2000 mg, 450 mg to 2000 mg, 500 mg to 2000 mg, 550 mg to 2000 mg, 600 mg to 2000 mg, 650 mg to 2000 mg, 700 mg to 2000 mg, 750 mg to 2000 mg, 800 mg to 2000 mg, 850 mg to 2000 mg, 900 mg to 2000 mg, 950 mg to 2000 mg, or 1000 mg to 2000 mg may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject every three months. In some embodiments, between about 225 mg to about 1000 mg is administered once every three months or less, e.g., about 900 mg is administered every three months intravenously in an infusion. An exemplary dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for one year, two years, three years, four years, or five years. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve.

In some embodiments, an antibody in a dose or amount of about 50 mg, 100 mg 150 mg, 200 mg, 225 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 675 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1000 mg, 1050 mg, 1100 mg, 1150 mg, 1200 mg, 1250 mg, 1300 mg, 1350 mg, 1400 mg, 1450 mg, 1500 mg, 1550 mg, 1600 mg, 1650 mg, 1700 mg, 1750 mg, 1800 mg, 1850 mg, 1900 mg, 1950 mg, 2000 mg, 2050 mg, 2100 mg, 2150 mg, 2200 mg, 2250 mg, 2300 mg, 2350 mg, 2400 mg, 2450 mg, 2500 mg, 2550 mg, 2600 mg, 2650 mg, 2700 mg, 2750 mg, 2800 mg, 2850 mg, 2900 mg, 2950 mg, 3000 mg, or more may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject every six months. In some embodiments, an antibody in a dose or amount of between about 0.1 mg to 5000 mg, 1 mg to 4000 mg, 10 mg to 3000 mg, 10 mg to 2000 mg, 100 mg to

2000 mg, 150 mg to 2000 mg, 200 mg to 2000 mg, 250 mg to 2000 mg, 300 mg to 2000 mg, 350 mg to 2000 mg, 400 mg to 2000 mg, 450 mg to 2000 mg, 500 mg to 2000 mg, 550 mg to 2000 mg, 600 mg to 2000 mg, 650 mg to 2000 mg, 700 mg to 2000 mg, 750 mg to 2000 mg, 800 mg to 2000 mg, 850 mg to 2000 mg, 900 mg to 2000 mg, 950 mg to 2000 mg, or 1000 mg to 2000 mg may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject every six months. In some embodiments, between 225 mg to 1000 mg is administered once every six months or less. An exemplary dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for one year, two years, three years, four years, or five years. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve.

In some embodiments, the frequency at which a dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) is administered to a subject (e.g., subcutaneously or intravenously) is, is at least, is less than, or is at most one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty time(s) per every quarter. As can be appreciated, a “quarter” can refer to a time period of a quarter year or may also refer to a calendar quarter such as a time period of January 1 – March 31, April 1 – June 30, July 1 – September 30, or October 1 – December 31. In some cases, a “quarter” may refer to a time period of approximately three months.

In some embodiments, an antibody in a dose or amount of about 50 mg, 100 mg 150 mg, 200 mg, 225 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 675 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1000 mg, 1050 mg, 1100 mg, 1150 mg, 1200 mg, 1250 mg, 1300 mg, 1350 mg, 1400 mg,

1450 mg, 1500 mg, 1550 mg, 1600 mg, 1650 mg, 1700 mg, 1750 mg, 1800 mg, 1850 mg, 1900 mg, 1950 mg, 2000 mg, 2050 mg, 2100 mg, 2150 mg, 2200 mg, 2250 mg, 2300 mg, 2350 mg, 2400 mg, 2450 mg, 2500 mg, 2550 mg, 2600 mg, 2650 mg, 2700 mg, 2750 mg, 2800 mg, 2850 mg, 2900 mg, 2950 mg, 3000 mg, or more may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject every quarter. In some embodiments, an antibody in a dose or amount of between about 0.1 mg to 5000 mg, 1 mg to 4000 mg, 10 mg to 3000 mg, 10 mg to 2000 mg, 100 mg to 2000 mg, 150 mg to 2000 mg, 200 mg to 2000 mg, 250 mg to 2000 mg, 300 mg to 2000 mg, 350 mg to 2000 mg, 400 mg to 2000 mg, 450 mg to 2000 mg, 500 mg to 2000 mg, 550 mg to 2000 mg, 600 mg to 2000 mg, 650 mg to 2000 mg, 700 mg to 2000 mg, 750 mg to 2000 mg, 800 mg to 2000 mg, 850 mg to 2000 mg, 900 mg to 2000 mg, 950 mg to 2000 mg, or 1000 mg to 2000 mg may be administered (e.g., subcutaneously or intravenously in an infusion) to a subject every quarter. An exemplary dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months. Yet another dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for one year, two years, three years, four years, or five years. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve.

In some embodiments, the frequency at which a dose or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) is administered is, is at least, is less than, or is at most about one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, or twenty time(s) per every year, every two years, every three years, every four years, or every five years. In some embodiments, the frequency at which an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist

antibody, monoclonal anti-CGRP antagonist antibody) is administered to a subject is less than one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-four or twenty-five dose(s) per year.

In some embodiments, an antibody in a dose or amount of about 50 mg, 100 mg 150 mg, 200 mg, 225 mg, 250 mg, 300 mg, 350 mg, 400 mg, 450 mg, 500 mg, 550 mg, 600 mg, 650 mg, 675 mg, 700 mg, 750 mg, 800 mg, 850 mg, 900 mg, 950 mg, 1000 mg, 1050 mg, 1100 mg, 1150 mg, 1200 mg, 1250 mg, 1300 mg, 1350 mg, 1400 mg, 1450 mg, 1500 mg, 1550 mg, 1600 mg, 1650 mg, 1700 mg, 1750 mg, 1800 mg, 1850 mg, 1900 mg, 1950 mg, 2000 mg, 2050 mg, 2100 mg, 2150 mg, 2200 mg, 2250 mg, 2300 mg, 2350 mg, 2400 mg, 2450 mg, 2500 mg, 2550 mg, 2600 mg, 2650 mg, 2700 mg, 2750 mg, 2800 mg, 2850 mg, 2900 mg, 2950 mg, 3000 mg, or more may be administered to a subject every once per year. In some embodiments, an antibody in a dose or amount of between about 0.1 mg to 5000 mg, 1 mg to 4000 mg, 10 mg to 3000 mg, 10 mg to 2000 mg, 100 mg to 2000 mg, 150 mg to 2000 mg, 200 mg to 2000 mg, 250 mg to 2000 mg, 300 mg to 2000 mg, 350 mg to 2000 mg, 400 mg to 2000 mg, 450 mg to 2000 mg, 500 mg to 2000 mg, 550 mg to 2000 mg, 600 mg to 2000 mg, 650 mg to 2000 mg, 700 mg to 2000 mg, 750 mg to 2000 mg, 800 mg to 2000 mg, 850 mg to 2000 mg, 900 mg to 2000 mg, 950 mg to 2000 mg, or 1000 mg to 2000 mg may be administered to a subject every once per year. In some embodiments, between about 450 mg and about 2000 mg is administered once every year or less.

In some embodiments, a method may comprise administering an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein to a subject on a plurality of days. Two, three, four, five, six, seven, eight or more days of the plurality of days may be more than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or more days apart. In some embodiments, two of the plurality of days are more than one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, twenty-one, twenty-two, twenty-three, twenty-

four, twenty-five, twenty-six, twenty-seven, twenty-eight, twenty-nine, thirty or more days apart. Moreover, in some embodiments, the amount of antibody administered on a first day of the plurality of days may be different (e.g., higher or lower) than the amount of the antibody administered on a second day.

In some embodiments, an initial dose (e.g., a loading dose) of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein may be administered to a subject, followed by administration of one or more additional doses at desired intervals. In some embodiments, the initial dose and one or more of the additional doses are the same dose. In some embodiments, the one or more additional doses are a different dose than the initial dose. In some embodiments, the initial dose and one or more of the additional doses are administered the same way, i.e., subcutaneously or intravenously. In some embodiments, the one or more additional doses are administered in a different way than the initial dose, e.g., the initial dose may be administered intravenously and the one or more additional doses may be administered subcutaneously. In some embodiments, the frequency at which the one or more additional doses are administered is constant (e.g., every month or every three months). In some embodiments, the frequency at which the one or more additional doses are administered is variable (e.g., one additional dose administered at one month following the initial dose, followed by another additional dose at three months following the initial dose). Any desirable and/or therapeutic regimen of initial loading dose, additional doses, and frequency (e.g., including those described herein) of additional doses may be used. An exemplary regimen includes an initial loading dose of about 675 mg anti-CGRP antagonist antibody administered subcutaneously, followed by subsequent maintenance doses of about 225 mg of the antibody administered subcutaneously at one month intervals. Yet another exemplary regimen includes an initial dose of about 900 mg anti-CGRP antagonist antibody administered intravenously in an infusion over about 60 minutes, followed by subsequent maintenance doses of about 900 mg anti-CGRP antagonist antibody administered intravenously in an infusion over about 60 minutes at three month intervals.

In some embodiments, an initial dose of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) of about 0.1 μg, 1 μg, 100 μg, 1 mg, 10 mg, 25 mg, 50 mg, 75 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 225 mg, 250 mg, 275 mg, 300 mg, 325 mg, 350 mg, 375 mg, 400 mg, 450 mg, 475 mg, 500 mg, 525 mg, 550 mg, 575 mg, 600 mg, 625 mg, 650 mg, 675 mg, 700 mg, 725 mg, 750 mg, 775 mg, 800 mg, 825 mg, 850 mg, 875 mg, 900 mg, 925 mg, 950 mg, 975 mg, 1000 mg, 1500 mg, 2000 mg, or about 3000 mg may be administered to a subject followed by one or more additional doses of the antibody of about 0.1 μg, 1 μg, 100 μg, 1 mg, 10 mg, 25 mg, 50 mg, 75 mg, 100 mg, 125 mg, 150 mg, 175 mg, 200 mg, 225 mg, 250 mg, 275 mg, 300 mg, 325 mg, 350 mg, 375 mg, 400 mg, 450 mg, 475 mg, 500 mg, 525 mg, 550 mg, 575 mg, 600 mg, 625 mg, 650 mg, 675 mg, 700 mg, 725 mg, 750 mg, 775 mg, 800 mg, 825 mg, 850 mg, 875 mg, 900 mg, 925 mg, 950 mg, 975 mg, 1000 mg, 1500 mg, 2000 mg, or about 3000 mg. An exemplary regimen includes an initial loading dose of about 675 mg anti-CGRP antagonist antibody administered subcutaneously, followed by subsequent maintenance doses of about 225 mg of the antibody administered subcutaneously at one month intervals. Yet another exemplary regimen includes an initial dose of about 900 mg anti-CGRP antagonist antibody administered intravenously in an infusion over about 60 minutes, followed by subsequent maintenance doses of about 900 mg anti-CGRP antagonist antibody administered intravenously in an infusion over about 60 minutes at three month intervals.

In some embodiments, a dose or amount of antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein may be divided into sub-doses and administered as multiple sub-doses, depending, for example, on the route of administration and/or particular formulation administered. For example, in cases where a dose is administered subcutaneously, the subcutaneous dose may be divided into multiple sub-doses and each sub-dose administered at a different site in order to avoid, for example, a larger, single subcutaneous injection at a single site. For example, an intravenous dose of 900 mg may be divided into four sub-doses of 225 mg each. As

another example, a subcutaneous dose of 675 mg may be divided into three sub-doses of 225 mg each and each 225 mg dose may be administered at a different site, which can help minimize the volume injected at each site. The division of sub-doses may be equal (e.g., three equal sub-doses) or may be unequal (e.g., three sub-doses, two of the sub-doses twice as large as the other sub-doses).

In some embodiments, the number of doses of antibody administered to a subject over the course of treatment may vary depending upon, for example, achieving reduced incidence of a post-traumatic headache and/or secondary symptom associated with a post-traumatic headache in the subject. For example, the number of doses administered over the course of treatment may be, may be at least, or may be at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or treatment may be given indefinitely. In some cases, treatment may be acute such that at most 1, 2, 3, 4, 5, or 6 doses are administered to a subject for treatment.

In some embodiments, a dose (or sub-dose) or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein may be formulated in a liquid formulation and administered (e.g., via subcutaneous injection, via intravenous injection) to a subject. In such cases, the volume of liquid formulation comprising antibody may vary depending upon, for example, the concentration of antibody in the liquid formulation, the desired dose of antibody, and/or the route of administration used. For example, the volume of liquid formulation comprising an antibody described herein and administered (e.g., via an injection, such as, for example, a subcutaneous injection or an intravenous infusion) to a subject may be from about 0.001 mL to about 10.0 mL, about 0.01 mL to about 5.0 mL, about 0.1 mL to about 5 mL, about 0.1 mL to about 3 mL, about 0.5 mL to about 2.5 mL, or about 1 mL to about 2.5 mL. For example, the volume of liquid formulation comprising an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein and administered (e.g., via an injection, such as, for example, a subcutaneous injection, or

an intravenous infusion) to a subject may be, may be at least, may be less than, or may be at most about 0.001, 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, or about 10.0 mL.

In some embodiments, a dose (or sub-dose) or amount of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein may be supplied in prefilled receptacles useful in administering antibody to a subject. Such prefilled receptacles may be designed for self-administration or for administration by another. For example, a dose (or sub-dose) or amount of antibody described herein may be supplied as a liquid formulation in pre-filled syringes, pre-filled syringes with a needle safety device, injection pens, or auto-injectors. In such examples, the pre-filled syringes may be designed for self-administration or for administration by another. In some cases, the pre-filled syringes or auto-injectors may be designed for subcutaneous administration and/or intravenous administration.

For the purpose of the present invention, the appropriate dosage of an antibody may depend on the antibody (or compositions thereof) employed, the type and severity of the secondary symptom, the type and severity of the (persistent) post-traumatic headache or other condition to be treated, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. Typically, the clinician will administer an antibody, until a dosage is reached that achieves the desired result. Dose and/or frequency can vary over course of treatment.

Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and

adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of post-traumatic headache or other condition. Alternatively, sustained continuous release formulations of antibodies may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

In one embodiment, dosages for an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein may be determined empirically in individuals who have been given one or more administration(s) of the antibody. Individuals are given incremental dosages of an antibody. To assess efficacy of an antibody, an indicator of the disease can be followed.

Administration of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) in accordance with the methods of the present invention can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an antibody may be essentially continuous over a preselected period of time or may be in a series of spaced dose, e.g., either before, during, or after developing (persistent) post-traumatic headache; before; during; before and after; during and after; before and during; or before, during, and after developing (persistent) post-traumatic headache. Administration can be before, during and/or after any event likely to give rise to (persistent) post-traumatic headache.

In some embodiments, more than one antibody may be present. At least one, at least two, at least three, at least four, at least five different, or more antibodies can be present. Generally, those antibodies may have complementary activities that do not adversely affect each other. An antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein can also be used in conjunction with other CGRP antagonists or CGRP receptor antagonists. For example, one or more of the following

CGRP antagonists may be used: an anti-sense molecule directed to a CGRP (including an anti-sense molecule directed to a nucleic acid encoding CGRP), a CGRP inhibitory compound, a CGRP structural analog, a dominant-negative mutation of a CGRP receptor that binds a CGRP, and an anti-CGRP receptor antibody. An antibody can also be used in conjunction with other agents that serve to enhance and/or complement the effectiveness of the agents.

Diagnosis or assessment of post-traumatic headache is well-established in the art. Assessment may be performed based on subjective measures, such as patient characterization of symptoms. In some embodiments, assessment of post-traumatic headache may be via headache hours, as described elsewhere herein. For example, assessment of post-traumatic headache may be in terms of daily headache hours, weekly headache hours, monthly headache hours and/or yearly headache hours. In some cases, headache hours may be as reported by the subject.

Treatment efficacy can be assessed by methods well-known in the art. For example, pain relief may be assessed. Accordingly, in some embodiments, pain relief is subjectively observed after 1, 2, or a few hours after administering an anti-CGRP antibody. In some embodiments, frequency of (persistent) post-traumatic headache attacks is subjectively observed after administering an anti-CGRP antibody.

In some embodiments, a method for preventing, treating, or reducing incidence of post-traumatic headache in a subject as described herein may reduce incidence of post-traumatic headache after a single administration of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein for an extended period of time. For example, incidence of (persistent) post-traumatic headache may be reduced for at least 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more days after a single administration.

In some embodiments, a method for treating or reducing incidence of post-traumatic headache in a subject as described herein may reduce the number of headache hours experienced by a subject from a pre-administration level after

administration of one or more doses of an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein to the subject. For example, daily headache hours experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 headache hours from a pre-administration level in the subject. In some cases, daily headache hours experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more relative to a pre-administration level in the subject. In another example, weekly headache hours experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 or more headache hours from a pre-administration level in the subject. In some cases, weekly headache hours experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99%, or more relative to a pre-administration level in the subject. In another example, monthly headache hours experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, or more headache hours from a pre-administration level. In some cases, weekly headache hours experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more relative to a pre-administration level in the subject.

In some embodiments, a method for treating or reducing incidence of (persistent) post-traumatic headache in a subject as described herein may reduce the number of headache days experienced by a subject from a pre-administration level after administration of one or more doses of an antibody (e.g., monoclonal antibody that

modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody) described herein to the subject. For example, weekly headache days experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, or 7 headache days from a pre-administration level in the subject. In some cases, weekly headache days experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5%, 1%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 99% or more relative to a pre-administration level in the subject. In another example, monthly headache days experienced by the subject after administering one or more doses of an antibody to the subject may be reduced by 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more headache days from a pre-administration level.

In some embodiments, a method may comprise administering to a subject one or more additional agent(s) simultaneously or sequentially with an antibody (e.g., monoclonal antibody that modulates the CGRP pathway, anti-CGRP antagonist antibody, monoclonal anti-CGRP antagonist antibody). In some embodiments, an additional agent may be an anti-headache medication such as an example anti-headache medication (e.g., 5-HT1 agonists, triptans, ergot alkaloids, opiates, β-adrenergic antagonists, NSAIDs) described elsewhere herein. In some embodiments, a therapeutic effect may be greater as compared to use of an antibody or one or more additional agent(s) alone. Accordingly, a synergistic effect between an antibody and the one or more additional agents may be achieved. In some embodiments, the one or more additional agent(s) may be taken by a subject prophylactically.

B. Anti-CGRP antagonist antibodies

In some embodiments, the methods of the invention use an antibody, which can be an anti-CGRP antagonist antibody. An anti-CGRP antagonist antibody can refer to any antibody molecule that blocks, suppresses or reduces (including significantly) CGRP biological activity, including downstream pathways mediated by CGRP signaling, such as receptor binding and/or elicitation of a cellular response to CGRP.

An anti-CGRP antagonist antibody can exhibit any one or more of the following characteristics: (a) bind to CGRP; (b) block CGRP from binding to its receptor(s); (c) block or decrease CGRP receptor activation (including, but not limited to, cAMP activation); (d) inhibit CGRP biological activity or downstream pathways mediated by CGRP signaling function; (e) prevent, ameliorate, or treat any aspect of post-traumatic headache; (f) increase clearance of CGRP; and (g) inhibit (reduce) CGRP synthesis, production or release. Anti-CGRP antagonist antibodies are known in the art. See e.g., Tan et al., Clin. Sci. (Lond). 89:565-73, 1995; Sigma (Missouri, US), product number C7113 (clone #4901); Plourde et al., Peptides 14:1225-1229, 1993.

In some embodiments, the antibody reacts with CGRP in a manner that inhibits CGRP, and/or the CGRP pathway, including downstream pathways mediated by the CGRP signaling function. In some embodiments, the anti-CGRP antagonist antibody recognizes human CGRP. In some embodiments, the anti-CGRP antagonist antibody binds to both human α-CGRP and β-CGRP. In some embodiments, the anti-CGRP antagonist antibody binds human and rat CGRP. In some embodiments, the anti-CGRP antagonist antibody binds the C-terminal fragment having amino acids 25-37 of CGRP. In some embodiments, the anti-CGRP antagonist antibody binds a C-terminal epitope within amino acids 25-37 of CGRP.

The antibodies useful in the present invention can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')2, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (e.g., a domain antibody), humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies).

In some embodiments, the anti-CGRP antagonist antibody is a monoclonal antibody. In some embodiments, the anti-CGRP antagonist antibody is humanized. In some embodiments, the antibody is human. In some embodiments, the anti-CGRP

antagonist antibody is antibody G1 (as described herein). In some embodiments, the anti-CGRP antagonist antibody comprises one or more CDR(s) (such as one, two, three, four, five, or, in some embodiments, all six CDRs) of antibody G1 or variants of G1 shown in Table 6. In still other embodiments, the anti-CGRP antagonist antibody comprises the amino acid sequence of the heavy chain variable region shown in Figure 5 (SEQ ID NO:1) and the amino acid sequence of the light chain variable region shown in Figure 5 (SEQ ID NO:2).

In some embodiments, the antibody comprises a light chain variable region (LCVR) and a heavy chain variable region (HCVR) selected from the groups consisting of: (a) LCVR17 (SEQ ID NO:58) and HCVR22 (SEQ ID NO:59); (b) LCVR18 (SEQ ID NO:60) and HCVR23 (SEQ ID NO:61); (c) LCVR19 (SEQ ID NO:62) and HCVR24 (SEQ ID NO:63); (d) LCVR20 (SEQ ID NO:64) and HCVR25 (SEQ ID NO:65); (e) LCVR21 (SEQ ID NO:66) and HCVR26 (SEQ ID NO:67); (f) LCVR27 (SEQ ID NO:68) and HCVR28 (SEQ ID NO:69); (g) LCVR29 (SEQ ID NO:70) and HCVR30 (SEQ ID NO:71); (h) LCVR31 (SEQ ID NO:72) and HCVR32 (SEQ ID NO:73); (i) LCVR33 (SEQ ID NO:74) and HCVR34 (SEQ ID NO:75); (j) LCVR35 (SEQ ID NO:76) and HCVR36 (SEQ ID NO:77); and (k) LCVR37 (SEQ ID NO:78) and HCVR38 (SEQ ID NO:79). Sequences of these regions are provided herein. Other examples of anti-CGRP antibodies are described in US20110305711 (SEQ ID NOs:5, 6, 7, 12, 16, 19, 24, 29, 34, and 39), US20120294802, US20120294797 (SEQ ID NOs:51-60), which are hereby incorporated by reference in their entireties. For example, antibodies with any of the following sequences may be used.

Ab6 Variable region Light chain (humanized) protein sequence (US20120294797)

QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASG VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIKR (SEQ ID NO:80)

Ab6 Light chain (humanized) Full length protein sequence (US20120294797)

QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASG
VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:81)

Ab6 Variable region heavy chain (humanized) protein sequence (US20120294797)

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGAT
YYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS
(SEQ ID NO:82)

Ab6 Heavy chain (humanized) Full length protein sequence - yeast produced (US20120294797)

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGAT
YYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSSAST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR
EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:83)

Ab6 Variable region Light chain (humanized) protein sequence CDRl (US20120294797)

QASQSVYHNTYLA (SEQ ID NO:84)

Ab6 Variable region Light chain (humanized) protein sequence CDR2 (US20120294797)

DASTLAS (SEQ ID NO:85)

Ab6 Variable region Light chain (humanized) protein sequence CDR3 (US20120294797)
LGSYDCTNGDCFV (SEQ ID NO:86)

Ab6 Variable region heavy chain (humanized) protein sequence CDRI (US20120294797)
GYYMN (SEQ ID NO:87)

Ab6 Variable region heavy chain (humanized) protein sequence CDR2 (US20120294797)
IGINGATYYASWAKG (SEQ ID NO:88)

Ab6 Variable region heavy chain (humanized) protein sequence CDR3 (US20120294797)
GDI (SEQ ID NO:89)

Light chain variable region protein sequence CDR3 (US20110305711)
QQGDALPPT (SEQ ID NO:90)

Light chain variable region protein sequence CDR1 (US20110305711)
RASKDISKYL (SEQ ID NO:91)

Light chain variable region protein sequence CDR2 (US20110305711)
YTSGYSH (SEQ ID NO:92)

Heavy chain variable region protein sequence CDR1 (US20110305711)
GYTFGNYWMQ (SEQ ID NO:93)

Heavy chain variable region protein sequence CDR2 (US20110305711)
AIYEGTGKTVYIQKFAD (SEQ ID NO:94)

Heavy chain variable region protein sequence CDR3 (US20110305711)
LSDYVSGFGY (SEQ ID NO:95)

Light chain variable region protein sequence (US20110305711)
DIQMTQSPSSLSASVGDRVTITCRASKDISKYLNWYQQKPGKAPKLLIYYTSGYHSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDALPPTFGGGTKVEIK (SEQ ID NO:96)

Heavy chain variable region protein sequence (US20110305711)
QVQLVQSGAEVKKPGSSVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT GKTVYIQKFADRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQGT TVTVSS (SEQ ID NO:97)

Light chain protein sequence (US20110305711)
DIQMTQSPSSLSASVGDRVTITCRASKDISKYLNWYQQKPGKAPKLLIYYTSGYHSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDALPPTFGGGTKVEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:98)

Heavy chain protein sequence (US20110305711)
QVQLVQSGAEVKKPGSSVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT GKTVYIQKFADRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQGT TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:99)

In some embodiments, the antibody comprises a modified constant region, such as a constant region that is immunologically inert described herein. In some embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624; PCT Application No. PCT/GB99/01441; and/or UK Patent Application No. 9809951.8. In other embodiments, the antibody comprises a human heavy chain IgG2 constant region comprising the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence). Eur. J. Immunol. (1999) 29:2613-2624. In some embodiments, the antibody comprises a constant region of IgG4 comprising the following mutations: E233F234L235 to P233V234A235. In still other embodiments, the constant region is aglycosylated for N-linked glycosylation. In some embodiments, the constant region is aglycosylated for N-linked glycosylation by mutating the oligosaccharide attachment residue (such as Asn297) and/or flanking residues that are part of the N-glycosylation recognition sequence in the constant region. In some embodiments, the constant region is aglycosylated for N-linked glycosylation. The constant region may be aglycosylated for N-linked glycosylation enzymatically or by expression in a glycosylation deficient host cell.

The binding affinity (K_D) of an anti-CGRP antagonist antibody to CGRP (such as human α-CGRP) can be about 0.02 to about 200 nM. In some embodiments, the binding affinity is any of about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, about 60 pM, about 50 pM, about 20 pM, about 15 pM, about 10 pM, about 5 pM, or about 2 pM. In some embodiments, the binding affinity is less than any of about 250 nM, about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, or about 50 pM.

One way of determining binding affinity of antibodies to CGRP is by measuring binding affinity of monofunctional Fab fragments of the antibody. To obtain monofunctional Fab fragments, an antibody (for example, IgG) can be cleaved with papain or expressed recombinantly. The affinity of an anti-CGRP Fab fragment of an antibody can be determined by surface plasmon resonance (Biacore3000™ surface plasmon resonance (SPR) system, Biacore, INC, Piscataway NJ) equipped with pre-immobilized streptavidin sensor chips (SA) using HBS-EP running buffer (0.01M

HEPES, pH 7.4, 0.15 NaCl, 3 mM EDTA, 0.005% v/v Surfactant P20). Biotinylated human CGRP (or any other CGRP) can be diluted into HBS-EP buffer to a concentration of less than 0.5 μg/mL and injected across the individual chip channels using variable contact times, to achieve two ranges of antigen density, either 50-200 response units (RU) for detailed kinetic studies or 800-1,000 RU for screening assays. Regeneration studies have shown that 25 mM NaOH in 25% v/v ethanol effectively removes the bound Fab while keeping the activity of CGRP on the chip for over 200 injections. Typically, serial dilutions (spanning concentrations of 0.1-10x estimated K_D) of purified Fab samples are injected for 1 min at 100 μL/minute and dissociation times of up to 2 hours are allowed. The concentrations of the Fab proteins are determined by ELISA and/or SDS-PAGE electrophoresis using a Fab of known concentration (as determined by amino acid analysis) as a standard. Kinetic association rates (k_{on}) and dissociation rates (k_{off}) are obtained simultaneously by fitting the data globally to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110) using the BIAevaluation program. Equilibrium dissociation constant (K_D) values are calculated as k_{off}/k_{on}. This protocol is suitable for use in determining binding affinity of an antibody to any CGRP, including human CGRP, CGRP of another mammalian (such as mouse CGRP, rat CGRP, primate CGRP), as well as different forms of CGRP (such as α and β form). Binding affinity of an antibody is generally measured at 25°C, but can also be measured at 37°C.

Antibodies, including anti-CGRP antagonist antibodies, may be made by any method known in the art. The route and schedule of immunization of the host animal are generally in keeping with established and conventional techniques for antibody stimulation and production, as further described herein. General techniques for production of human and mouse antibodies are known in the art and are described herein.

It is contemplated that any mammalian subject including humans or antibody producing cells therefrom can be manipulated to serve as the basis for production of mammalian, including human, hybridoma cell lines. Typically, the host animal is

inoculated intraperitoneally, intramuscularly, orally, subcutaneously, intraplantar, and/or intradermally with an amount of immunogen, including as described herein.

Hybridomas can be prepared from the lymphocytes and immortalized myeloma cells using the general somatic cell hybridization technique of Kohler, B. and Milstein, C. (1975) Nature 256:495-497 or as modified by Buck, D. W., et al., In Vitro, 18:377-381 (1982). Available myeloma lines, including but not limited to X63-Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, Calif., USA, may be used in the hybridization. Generally, the technique involves fusing myeloma cells and lymphoid cells using a fusogen such as polyethylene glycol, or by electrical means well known to those skilled in the art. After the fusion, the cells are separated from the fusion medium and grown in a selective growth medium, such as hypoxanthine-aminopterin-thymidine (HAT) medium, to eliminate unhybridized parent cells. Any of the media described herein, supplemented with or without serum, can be used for culturing hybridomas that secrete monoclonal antibodies. As another alternative to the cell fusion technique, EBV immortalized B cells may be used to produce monoclonal antibodies (e.g., monoclonal the anti-CGRP antibodies) of the subject invention. The hybridomas are expanded and subcloned, if desired, and supernatants are assayed for anti-immunogen activity by conventional immunoassay procedures (e.g., radioimmunoassay, enzyme immunoassay, or fluorescence immunoassay).

Hybridomas that may be used as source of antibodies encompass all derivatives, progeny cells of the parent hybridomas that produce monoclonal antibodies specific for CGRP, or a portion thereof.

Hybridomas that produce such antibodies may be grown in vitro or in vivo using known procedures. The monoclonal antibodies may be isolated from the culture media or body fluids, by conventional immunoglobulin purification procedures such as ammonium sulfate precipitation, gel electrophoresis, dialysis, chromatography, and ultrafiltration, if desired. Undesired activity if present, can be removed, for example, by running the preparation over adsorbents made of the immunogen attached to a solid phase and eluting or releasing the desired antibodies off the immunogen. Immunization of a host animal with a human CGRP, or a fragment containing the target amino acid

sequence conjugated to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaradehyde, succinic anhydride, SOCl2, or R1N=C=NR, where R and R1 are different alkyl groups, can yield a population of antibodies (e.g., monoclonal antibodies).

If desired, an antibody (e.g., monoclonal or polyclonal anti-CGRP antagonist antibody) of interest may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use. In an alternative, the polynucleotide sequence may be used for genetic manipulation to "humanize" the antibody or to improve the affinity, or other characteristics of the antibody. For example, the constant region may be engineered to more resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. It may be desirable to genetically manipulate the antibody sequence to obtain greater affinity to CGRP and greater efficacy in inhibiting CGRP. It will be apparent to one of skill in the art that one or more polynucleotide changes can be made to the anti-CGRP antagonist antibody and still maintain its binding ability to CGRP.

Humanizing a monoclonal antibody can comprise four general steps. These are: (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody, i.e., deciding which antibody framework region to use during the humanizing process (3) the actual humanizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patent Nos. 4,816,567; 5,807,715; 5,866,692; 6,331,415; 5,530,101; 5,693,761; 5,693,762; 5,585,089; and 6,180,370.

A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent V regions and their associated

complementarity determining regions (CDRs) fused to human constant domains. See, for example, Winter et al., Nature 349:293-299 (1991), Lobuglio et al., Proc. Nat. Acad. Sci. USA 86:4220-4224 (1989), Shaw et al., J Immunol. 138:4534-4538 (1987), and Brown et al., Cancer Res. 47:3577-3583 (1987). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody constant domain. See, for example, Riechmann et al., Nature 332:323-327 (1988), Verhoeyen et al. Science 239:1534-1536 (1988), and Jones et al., Nature 321:522-525 (1986). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 0519596. These "humanized" molecules are designed to minimize unwanted immunological response toward rodent anti-human antibody molecules which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. For example, the antibody constant region can be engineered such that it is immunologically inert (e.g., does not trigger complement lysis). See, e.g., PCT Publication No. PCT/GB99/01441; UK Patent Application No. 9809951.8. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty et al., Nucl. Acids Res. 19:2471-2476 (1991) and in U.S. Patent Nos. 6,180,377; 6,054,297; 5,997,867; 5,866,692; 6,210,671; and 6,350,861; and in PCT Publication No. WO 01/27160.

In yet another alternative, fully human antibodies may be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are XENOMOUSE™ from Abgenix, Inc. (Fremont, CA) and HuMAb-Mouse® and TC Mouse™ from Medarex, Inc. (Princeton, NJ).

In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. In another alternative, antibodies may be made recombinantly by phage display technology. See, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; and 6,265,150; and Winter et al., Annu. Rev.

Immunol. 12:433-455 (1994). Alternatively, the phage display technology (McCafferty et al., Nature 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for review see, e.g., Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Mark et al., J. Mol. Biol. 222:581-597 (1991), or Griffith et al., EMBO J. 12:725-734 (1993). In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as “chain shuffling.” Marks, et al., Bio/Technol. 10:779-783 (1992)). In this method, the affinity of “primary” human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the pM-nM range. A strategy for making very large phage antibody repertoires (also known as “the mother-of-all libraries”) has been described by Waterhouse et al., Nucl.

Acids Res. 21:2265-2266 (1993). Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable regions capable of restoring a functional antigen-binding site, i.e., the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT Publication No. WO 93/06213, published April 1, 1993). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

It is apparent that although the above discussion pertains to humanized antibodies, the general principles discussed are applicable to customizing antibodies for use, for example, in dogs, cats, primate, equines and bovines. It is further apparent that one or more aspects of humanizing an antibody described herein may be combined, e.g., CDR grafting, framework mutation and CDR mutation.

Antibodies may be made recombinantly by first isolating the antibodies and antibody producing cells from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (e.g., CHO cells). Another method which may be employed is to express the antibody sequence in plants (e.g., tobacco) or transgenic milk. Methods for expressing antibodies recombinantly in plants or milk have been disclosed. See, for example, Peeters, et al. Vaccine 19:2756 (2001); Lonberg, N. and D. Huszar Int. Rev. Immunol 13:65 (1995); and Pollock, et al., J Immunol Methods 231:147(1999). Methods for making derivatives of antibodies, e.g., humanized, single chain, etc. are known in the art.

Immunoassays and flow cytometry sorting techniques such as fluorescence activated cell sorting (FACS) can also be employed to isolate antibodies that are specific for CGRP.

The antibodies can be bound to many different carriers. Carriers can be active and/or inert. Examples of well-known carriers include polypropylene, polystyrene, polyethylene, dextran, nylon, amylases, glass, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation. In some embodiments, the carrier comprises a moiety that targets the myocardium.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors (such as expression vectors disclosed in PCT Publication No. WO 87/04462), which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. See, e.g., PCT Publication No. WO 87/04462. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison et al., Proc. Nat. Acad. Sci. 81:6851 (1984), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, “chimeric” or “hybrid” antibodies are prepared that have the binding specificity of an anti-CGRP monoclonal antibody herein.

Antibodies (e.g., anti-CGRP antagonist antibodies) and polypeptides derived from antibodies can be identified or characterized using methods known in the art, whereby reduction, amelioration, or neutralization of a CGRP biological activity is detected and/or measured. For example, anti-CGRP antagonist antibody can also be identified by incubating a candidate agent with CGRP and monitoring any one or more of the following characteristics: (a) bind to CGRP; (b) block CGRP from binding to its receptor(s); (c) block or decrease CGRP receptor activation (including cAMP activation);

(d) inhibit CGRP biological activity or downstream pathways mediated by CGRP signaling function; (e) prevent, ameliorate, or treat any aspect of post-traumatic headache; (f) increase clearance of CGRP; and (g) inhibit (reduce) CGRP synthesis, production or release. In some embodiments, an anti-CGRP antagonist antibody or polypeptide is identified by incubating a candidate agent with CGRP and monitoring binding and/or attendant reduction or neutralization of a biological activity of CGRP. The binding assay may be performed with purified CGRP polypeptide(s), or with cells naturally expressing, or transfected to express, CGRP polypeptide(s). In one embodiment, the binding assay is a competitive binding assay, where the ability of a candidate antibody to compete with a known anti-CGRP antagonist for CGRP binding is evaluated. The assay may be performed in various formats, including the ELISA format. In other embodiments, an anti-CGRP antagonist antibody is identified by incubating a candidate agent with CGRP and monitoring binding and attendant inhibition of CGRP receptor activation expressed on the surface of a cell. In some embodiments, an anti-CGRP receptor antibody can be used in any of the methods described herein. For example, anti-CGRP receptor antibodies, as described in US20100172895 and U.S. Patent No. 9,102,731, which are hereby incorporated by reference in their entireties, may be used.

Following initial identification, the activity of a candidate antibody (e.g., anti-CGRP antagonist antibody) can be further confirmed and refined by bioassays, known to test the targeted biological activities. Alternatively, bioassays can be used to screen candidates directly. For example, CGRP promotes a number of measurable changes in responsive cells. These include, but are not limited to, stimulation of cAMP in the cell (e.g., SK-N-MC cells). Antagonist activity may also be measured using animal models, such as measuring skin vasodilatation induced by stimulation of the rat saphenous nerve. Escott et al., Br. J. Pharmacol. 110: 772-776, 1993. Animal models of post-traumatic headaches may further be used for testing efficacy of antagonist antibodies or polypeptides. Reuter, et al., Functional Neurology (15) Suppl.3, 2000. Some of the methods for identifying and characterizing anti-CGRP antagonist antibody or polypeptide are described in detail in the Examples.

Antibodies, including anti-CGRP antagonist antibodies, may be characterized using methods well known in the art. For example, one method is to identify the epitope to which it binds, or "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. In an additional example, epitope mapping can be used to determine the sequence to which an anti-CGRP antagonist antibody binds. Epitope mapping is commercially available from various sources, for example, Pepscan Systems (Edelhertweg 15, 8219 PH Lelystad, The Netherlands). The epitope can be a linear epitope, i.e., contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch. Peptides of varying lengths (e.g., at least 4-6 amino acids long) can be isolated or synthesized (e.g., recombinantly) and used for binding assays with an anti-CGRP antagonist antibody. In another example, the epitope to which the anti-CGRP antagonist antibody binds can be determined in a systematic screening by using overlapping peptides derived from the CGRP sequence and determining binding by the anti-CGRP antagonist antibody. According to the gene fragment expression assays, the open reading frame encoding CGRP is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of CGRP with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled CGRP fragments is then determined by immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. In an additional example,

mutagenesis of an antigen binding domain, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding. For example, domain swapping experiments can be performed using a mutant CGRP in which various fragments of the CGRP polypeptide have been replaced (swapped) with sequences from a closely related, but antigenically distinct protein (such as another member of the neurotrophin protein family). By assessing binding of the antibody to the mutant CGRP, the importance of the particular CGRP fragment to antibody binding can be assessed.

Yet another method which can be used to characterize an antibody, including an anti-CGRP antagonist antibody, is to use competition assays with other antibodies known to bind to the same antigen, i.e., various fragments on CGRP, to determine if the anti-CGRP antagonist antibody binds to the same epitope as other antibodies. Competition assays are well known to those of skill in the art.

An expression vector can be used to direct expression of an antibody, including an anti-CGRP antagonist antibody. One skilled in the art is familiar with administration of expression vectors to obtain expression of an exogenous protein in vivo. See, e.g., U.S. Patent Nos. 6,436,908; 6,413,942; and 6,376,471. Administration of expression vectors includes local or systemic administration, including injection, oral administration, particle gun or catheterized administration, and topical administration. In another embodiment, the expression vector is administered directly to the sympathetic trunk or ganglion, or into a coronary artery, atrium, ventrical, or pericardium.

Targeted delivery of therapeutic compositions containing an expression vector, or subgenomic polynucleotides can also be used. Receptor-mediated DNA delivery techniques are described in, for example, Findeis et al., Trends Biotechnol. (1993) 11:202; Chiou et al., Gene Therapeutics: Methods and Applications of Direct Gene Transfer (J.A. Wolff, ed.) (1994); Wu et al., J. Biol. Chem. (1988) 263:621; Wu et al., J. Biol. Chem. (1994) 269:542; Zenke et al., Proc. Natl. Acad. Sci. USA (1990) 87:3655; Wu et al., J. Biol. Chem. (1991) 266:338. Therapeutic compositions containing a polynucleotide are administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng

to about 50 mg, about 1 μg to about 2 mg, about 5 μg to about 500 μg, and about 20 μg to about 100 μg of DNA can also be used during a gene therapy protocol. The therapeutic polynucleotides and polypeptides can be delivered using gene delivery vehicles. The gene delivery vehicle can be of viral or non-viral origin (see generally, Jolly, Cancer Gene Therapy (1994) 1:51; Kimura, Human Gene Therapy (1994) 5:845; Connelly, Human Gene Therapy (1995) 1:185; and Kaplitt, Nature Genetics (1994) 6:148). Expression of such coding sequences can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence can be either constitutive or regulated.

Viral-based vectors for delivery of a desired polynucleotide and expression in a desired cell are well known in the art. Exemplary viral-based vehicles include, but are not limited to, recombinant retroviruses (see, e.g., PCT Publication Nos. WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; WO 93/11230; WO 93/10218; WO 91/02805; U.S. Patent Nos. 5, 219,740 and 4,777,127; GB Patent No. 2,200,651; and EP Patent No. 0 345 242), alphavirus-based vectors (e.g., Sindbis virus vectors, Semliki forest virus (ATCC VR-67; ATCC VR-1247), Ross River virus (ATCC VR-373; ATCC VR-1246) and Venezuelan equine encephalitis virus (ATCC VR-923; ATCC VR-1250; ATCC VR 1249; ATCC VR-532)), and adeno-associated virus (AAV) vectors (see, e.g., PCT Publication Nos. WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655). Administration of DNA linked to killed adenovirus as described in Curiel, Hum. Gene Ther. (1992) 3:147 can also be employed.

Non-viral delivery vehicles and methods can also be employed, including, but not limited to, polycationic condensed DNA linked or unlinked to killed adenovirus alone (see, e.g., Curiel, Hum. Gene Ther. (1992) 3:147); ligand-linked DNA (see, e.g., Wu, J. Biol. Chem. (1989) 264:16985); eukaryotic cell delivery vehicles cells (see, e.g., U.S. Patent No. 5,814,482; PCT Publication Nos. WO 95/07994; WO 96/17072; WO 95/30763; and WO 97/42338) and nucleic charge neutralization or fusion with cell membranes. Naked DNA can also be employed. Exemplary naked DNA introduction methods are described in PCT Publication No. WO 90/11092 and U.S. Patent No. 5,580,859. Liposomes that can act as gene delivery vehicles are described in U.S.

Patent No. 5,422,120; PCT Publication Nos. WO 95/13796; WO 94/23697; WO 91/14445; and EP 0524968. Additional approaches are described in Philip, Mol. Cell Biol. (1994) 14:2411, and in Woffendin, Proc. Natl. Acad. Sci. (1994) 91:1581.

C. Antibody G1 and related antibodies, polypeptides, polynucleotides, vectors and host cells

This invention encompasses compositions, including pharmaceutical compositions, comprising antibody G1 and its variants shown in Table 6 or polypeptide derived from antibody G1 and its variants shown in Table 6; and polynucleotides comprising sequences encoding G1 and its variants or the polypeptide. In some embodiments, compositions comprise one or more antibodies or polypeptides (which may or may not be an antibody) that bind to CGRP, and/or one or more polynucleotides comprising sequences encoding one or more antibodies or polypeptides that bind to CGRP. These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients including buffers, which are well known in the art.

In some embodiments, the anti-CGRP antagonist antibodies and polypeptides of the invention are characterized by any (one or more) of the following characteristics: (a) bind to CGRP; (b) block CGRP from binding to its receptor(s); (c) block or decrease CGRP receptor activation (including cAMP activation); (d) inhibit CGRP biological activity or downstream pathways mediated by CGRP signaling function; (e) prevent, ameliorate, or treat any aspect of post-traumatic headache; (f) increase clearance of CGRP; and (g) inhibit (reduce) CGRP synthesis, production or release.

In some embodiments, the invention provides any of the following, or compositions (including pharmaceutical compositions) comprising any of the following: (a) antibody G1 or its variants shown in Table 6; (b) a fragment or a region of antibody G1 or its variants shown in Table 6; (c) a light chain of antibody G1 or its variants shown in Table 6; (d) a heavy chain of antibody G1 or its variants shown in Table 6; (e) one or more variable region(s) from a light chain and/or a heavy chain of antibody G1 or its variants shown in Table 6; (f) one or more CDR(s) (one, two, three, four, five or six

CDRs) of antibody G1 or its variants shown in Table 6; (g) CDR H3 from the heavy chain of antibody G1; (h) CDR L3 from the light chain of antibody G1 or its variants shown in Table 6; (i) three CDRs from the light chain of antibody G1 or its variants shown in Table 6; (j) three CDRs from the heavy chain of antibody G1 or its variants shown in Table 6; (k) three CDRs from the light chain and three CDRs from the heavy chain, of antibody G1 or its variants shown in Table 6; and (l) an antibody comprising any one of (b) through (k). In some embodiments, the invention also provides polypeptides comprising any one or more of the above.

The CDR portions of antibody G1 (including Chothia and Kabat CDRs) are diagrammatically depicted in Figure 5. Determination of CDR regions is well within the skill of the art. It is understood that in some embodiments, CDRs can be a combination of the Kabat and Chothia CDR (also termed "combined CDRs" or "extended CDRs"). In some embodiments, the CDRs are the Kabat CDRs. In other embodiments, the CDRs are the Chothia CDRs. In other words, in embodiments with more than one CDR, the CDRs may be any of Kabat, Chothia, combination CDRs, or combinations thereof.

In some embodiments, the invention provides a polypeptide (which may or may not be an antibody) which comprises at least one CDR, at least two, at least three, or at least four, at least five, or all six CDRs that are substantially identical to at least one CDR, at least two, at least three, at least four, at least five or all six CDRs of G1 or its variants shown in Table 6. Other embodiments include antibodies which have at least two, three, four, five, or six CDR(s) that are substantially identical to at least two, three, four, five or six CDRs of G1 or derived from G1. In some embodiments, the at least one, two, three, four, five, or six CDR(s) are at least about 85%, 86%, 87%, 88%, 89%, 90%, 95%, 96%, 97%, 98%, or 99% identical to at least one, two, three, four, five or six CDRs of G1 or its variants shown in Table 6. It is understood that, for purposes of this invention, binding specificity and/or overall activity is generally retained, although the extent of activity may vary compared to G1 or its variants shown in Table 6 (may be greater or lesser).

In some embodiments, the invention also provides a polypeptide (which may or may not be an antibody) which comprises an amino acid sequence of G1 or its variants

shown in Table 6 that has any of the following: at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, at least about 20 contiguous amino acids, at least about 25 contiguous amino acids, at least about 30 contiguous amino acids of a sequence of G1 or its variants shown in Table 6, wherein at least 3 of the amino acids are from a variable region of G1 (Figure 5) or its variants shown in Table 6. In one embodiment, the variable region is from a light chain of G1. In another embodiment, the variable region is from a heavy chain of G1. An exemplary polypeptide has contiguous amino acid (lengths described above) from both the heavy and light chain variable regions of G1. In another embodiment, the 5 (or more) contiguous amino acids are from a complementarity determining region (CDR) of G1 shown in Figure 5. In some embodiments, the contiguous amino acids are from a variable region of G1.

The binding affinity (K_D) of an anti-CGRP antagonist antibody and polypeptide to CGRP (such as human α-CGRP) can be about 0.06 to about 200 nM. In some embodiments, the binding affinity is any of about 200 nM, 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, about 60 pM, about 50 pM, about 20 pM, about 15 pM, about 10 pM, about 5 pM, or about 2 pM. In some embodiments, the binding affinity is less than any of about 250 nM, about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, or about 50 pM.

In some embodiments, the invention also provides methods of making any of these antibodies or polypeptides. The antibodies of this invention can be made by procedures known in the art. The polypeptides can be produced by proteolytic or other degradation of the antibodies, by recombinant methods (i.e., single or fusion polypeptides) as described above or by chemical synthesis. Polypeptides of the antibodies, especially shorter polypeptides up to about 50 amino acids, are conveniently made by chemical synthesis. Methods of chemical synthesis are known in the art and are commercially available. For example, an antibody could be produced by an automated polypeptide synthesizer employing the solid phase method. See also, U.S. Patent Nos. 5,807,715; 4,816,567; and 6,331,415.

In another alternative, the antibodies can be made recombinantly using procedures that are well known in the art. In one embodiment, a polynucleotide comprises a sequence encoding the heavy chain and/or the light chain variable regions of antibody G1 shown in SEQ ID NO:9 and SEQ ID NO:10. In another embodiment, the polynucleotide comprising the nucleotide sequence shown in SEQ ID NO:9 and SEQ ID NO:10 are cloned into one or more vectors for expression or propagation. The sequence encoding the antibody of interest may be maintained in a vector in a host cell and the host cell can then be expanded and frozen for future use. Vectors (including expression vectors) and host cells are further described herein.

In some embodiments, the invention also encompasses single chain variable region fragments ("scFv") of antibodies of this invention, such as G1. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide. Bird et al. (1988) Science 242:423-426. An example of a linking peptide is (GGGGS)3 (SEQ ID NO:57) which bridges approximately 3.5 nm between the carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used. Bird et al. (1988). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding

sites (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123).

For example, bispecific antibodies, monoclonal antibodies that have binding specificities for at least two different antigens, can be prepared using the antibodies disclosed herein. Methods for making bispecific antibodies are known in the art (see, e.g., Suresh et al., 1986, Methods in Enzymology 121:210). Traditionally, the recombinant production of bispecific antibodies was based on the coexpression of two immunoglobulin heavy chain-light chain pairs, with the two heavy chains having different specificities (Millstein and Cuello, 1983, Nature 305, 537-539).

According to one approach to making bispecific antibodies, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CH1), containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In one approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure, with an immunoglobulin light chain in only one half of the bispecific molecule, facilitates the separation of the desired bispecific

compound from unwanted immunoglobulin chain combinations. This approach is described in PCT Publication No. WO 94/04690, published March 3, 1994.

Heteroconjugate antibodies, comprising two covalently joined antibodies, are also within the scope of the invention. Such antibodies have been used to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373; EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents and techniques are well known in the art, and are described in U.S. Patent No. 4,676,980.

Chimeric or hybrid antibodies also may be prepared in vitro using known methods of synthetic protein chemistry, including those involving cross-linking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

Humanized antibody comprising one or more CDRs of antibody G1 or its variants shown in Table 6, or one or more CDRs derived from antibody G1 or its variants shown in Table 6 can be made using any methods known in the art. For example, four general steps may be used to humanize a monoclonal antibody.

In some embodiments, the invention encompasses modifications to antibody G1 or its variants shown in Table 6, including functionally equivalent antibodies which do not significantly affect their properties and variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence of antibody G1 or its variants shown in Table 6 may be mutated to obtain an antibody with the desired binding affinity to CGRP. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Modification of polypeptides is exemplified in the Examples. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the serum half-life of the antibody.

Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1: Amino Acid Substitutions

Original Residue	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn

Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

(1) Non-polar: Norleucine, Met, Ala, Val, Leu, Ile;

(2) Polar without charge: Cys, Ser, Thr, Asn, Gln;

(3) Acidic (negatively charged): Asp, Glu;

(4) Basic (positively charged): Lys, Arg;

(5) Residues that influence chain orientation: Gly, Pro; and

(6) Aromatic: Trp, Tyr, Phe, His.

Non-conservative substitutions are made by exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant cross-linking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability, particularly where the antibody is an antibody fragment such as an Fv fragment.

Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in

the variable region can alter binding affinity and/or specificity. In some embodiments, no more than one to five conservative amino acid substitutions are made within a CDR domain. In other embodiments, no more than one to three conservative amino acid substitutions are made within a CDR domain. In still other embodiments, the CDR domain is CDR H3 and/or CDR L3.

Modifications also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, 1997, Chem. Immunol. 65:111-128; Wright and Morrison, 1997, TibTECH 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., 1996, Mol. Immunol. 32:1311-1318; Wittwe and Howard, 1990, Biochem. 29:4175-4180) and the intramolecular interaction between portions of the glycoprotein, which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, supra; Wyss and Wagner, 1996, Current Opin. Biotech. 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al., 1999, Mature Biotech. 17:176-180).

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, asparagine-X-threonine, and asparagine-X-cysteine, 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 the 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).

The glycosylation pattern of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g., antibodies, as potential therapeutics is rarely the native cell, variations in the glycosylation pattern of the antibodies can be expected (see, e.g., Hse et al., 1997, J. Biol. Chem. 272:9062-9070).

In addition to the choice of host cells, factors that affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U.S. Patent Nos. 5,047,335; 5,510,261, and 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H), N-glycosidase F, endoglycosidase F1, endoglycosidase F2, endoglycosidase F3. In addition, the recombinant host cell can be genetically engineered to be defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay. Modified G1 polypeptides can be made using established procedures in the art and can be screened using standard assays known in the art, some of which are described below and in the Examples.

In some embodiments of the invention, the antibody comprises a modified constant region, such as a constant region that is immunologically inert or partially inert, e.g., does not trigger complement mediated lysis, does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC), or does not activate microglia; or have reduced activities (compared to the unmodified antibody) in any one or more of the following: triggering complement mediated lysis, stimulating antibody-dependent cell mediated cytotoxicity (ADCC), or activating microglia. Different modifications of the constant region may be used to achieve optimal level and/or combination of effector functions. See, for example, Morgan et al., Immunology 86:319-324 (1995); Lund et al., J. Immunology 157:4963-9 157:4963-4969 (1996); Idusogie et al., J. Immunology 164:4178-4184 (2000); Tao et al., J. Immunology 143: 2595-2601 (1989); and Jefferis et al., Immunological Reviews 163:59-76 (1998). In some embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624; PCT Application No. PCT/GB99/01441; and/or UK Patent Application No. 9809951.8. In other embodiments, the antibody comprises a human heavy chain IgG2 constant region comprising the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence). Eur. J. Immunol. (1999) 29:2613-2624. In still other embodiments, the constant region is aglycosylated for N-linked glycosylation. In some embodiments, the constant region is aglycosylated for N-linked glycosylation by mutating the glycosylated amino acid residue or flanking residues that are part of the N-glycosylation recognition sequence in the constant region. For example, N-glycosylation site N297 may be mutated to A, Q, K, or H. See, Tao et al., J. Immunology 143: 2595-2601 (1989); and Jefferis et al., Immunological Reviews 163:59-76 (1998). In some embodiments, the constant region is aglycosylated for N-linked glycosylation. The constant region may be aglycosylated for N-linked glycosylation enzymatically (such as removing carbohydrate by enzyme PNGase), or by expression in a glycosylation deficient host cell.

Other antibody modifications include antibodies that have been modified as described in PCT Publication No. WO 99/58572, published November 18, 1999. These antibodies comprise, in addition to a binding domain directed at the target molecule, an

effector domain having an amino acid sequence substantially homologous to all or part of a constant domain of a human immunoglobulin heavy chain. These antibodies are capable of binding the target molecule without triggering significant complement dependent lysis, or cell-mediated destruction of the target. In some embodiments, the effector domain is capable of specifically binding FcRn and/or FcγRIIb. These are typically based on chimeric domains derived from two or more human immunoglobulin heavy chain C_H2 domains. Antibodies modified in this manner are particularly suitable for use in chronic antibody therapy, to avoid inflammatory and other adverse reactions to conventional antibody therapy.

In some embodiments, the invention includes affinity matured embodiments. For example, affinity matured antibodies can be produced by procedures known in the art (Marks et al., 1992, Bio/Technology, 10:779-783; Barbas et al., 1994, Proc Nat. Acad. Sci, USA 91:3809-3813; Schier et al., 1995, Gene, 169:147-155; Yelton et al., 1995, J. Immunol., 155:1994-2004; Jackson et al., 1995, J. Immunol., 154(7):3310-9; Hawkins et al, 1992, J. Mol. Biol., 226:889-896; and WO2004/058184).

The following methods may be used for adjusting the affinity of an antibody and for characterizing a CDR. One way of characterizing a CDR of an antibody and/or altering (such as improving) the binding affinity of a polypeptide, such as an antibody, termed "library scanning mutagenesis". Generally, library scanning mutagenesis works as follows. One or more amino acid positions in the CDR are replaced with two or more (such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids using art recognized methods. This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of two or more members (if two or more amino acids are substituted at every position). Generally, the library also includes a clone comprising the native (unsubstituted) amino acid. A small number of clones, e.g., about 20-80 clones (depending on the complexity of the library), from each library are screened for binding affinity to the target polypeptide (or other binding target), and candidates with increased, the same, decreased or no binding are identified. Methods for determining binding affinity are well-known in the art. Binding affinity may be determined using Biacore

surface plasmon resonance analysis, which detects differences in binding affinity of about 2-fold or greater. Biacore is particularly useful when the starting antibody already binds with a relatively high affinity, for example a K_D of about 10 nM or lower. Screening using Biacore surface plasmon resonance is described in the Examples, herein.

Binding affinity may be determined using Kinexa Biocensor, scintillation proximity assays, ELISA, ORIGEN immunoassay (IGEN), fluorescence quenching, fluorescence transfer, and/or yeast display. Binding affinity may also be screened using a suitable bioassay.

In some embodiments, every amino acid position in a CDR is replaced (in some embodiments, one at a time) with all 20 natural amino acids using art recognized mutagenesis methods (some of which are described herein). This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of 20 members (if all 20 amino acids are substituted at every position).

In some embodiments, the library to be screened comprises substitutions in two or more positions, which may be in the same CDR or in two or more CDRs. Thus, the library may comprise substitutions in two or more positions in one CDR. The library may comprise substitution in two or more positions in two or more CDRs. The library may comprise substitution in 3, 4, 5, or more positions, said positions found in two, three, four, five or six CDRs. The substitution may be prepared using low redundancy codons. See, e.g., Table 2 of Balint et al., (1993) Gene 137(1):109-18).

The CDR may be CDRH3 and/or CDRL3. The CDR may be one or more of CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, and/or CDRH3. The CDR may be a Kabat CDR, a Chothia CDR, or an extended CDR.

Candidates with improved binding may be sequenced, thereby identifying a CDR substitution mutant which results in improved affinity (also termed an "improved" substitution). Candidates that bind may also be sequenced, thereby identifying a CDR substitution which retains binding.

Multiple rounds of screening may be conducted. For example, candidates (each comprising an amino acid substitution at one or more position of one or more CDR) with improved binding are also useful for the design of a second library containing at least the original and substituted amino acid at each improved CDR position (i.e., amino acid position in the CDR at which a substitution mutant showed improved binding). Preparation, and screening or selection of this library is discussed further below.

Library scanning mutagenesis also provides a means for characterizing a CDR, in so far as the frequency of clones with improved binding, the same binding, decreased binding or no binding also provide information relating to the importance of each amino acid position for the stability of the antibody-antigen complex. For example, if a position of the CDR retains binding when changed to all 20 amino acids, that position is identified as a position that is unlikely to be required for antigen binding. Conversely, if a position of CDR retains binding in only a small percentage of substitutions, that position is identified as a position that is important to CDR function. Thus, the library scanning mutagenesis methods generate information regarding positions in the CDRs that can be changed to many different amino acids (including all 20 amino acids), and positions in the CDRs which cannot be changed or which can only be changed to a few amino acids.

Candidates with improved affinity may be combined in a second library, which includes the improved amino acid, the original amino acid at that position, and may further include additional substitutions at that position, depending on the complexity of the library that is desired, or permitted using the desired screening or selection method. In addition, if desired, adjacent amino acid position can be randomized to at least two or more amino acids. Randomization of adjacent amino acids may permit additional conformational flexibility in the mutant CDR, which may in turn, permit or facilitate the introduction of a larger number of improving mutations. The library may also comprise substitution at positions that did not show improved affinity in the first round of screening.

The second library is screened or selected for library members with improved and/or altered binding affinity using any method known in the art, including screening

using Biacore surface plasmon resonance analysis, and selection using any method known in the art for selection, including phage display, yeast display, and ribosome display.

In some embodiments, the invention also encompasses fusion proteins comprising one or more fragments or regions from the antibodies (such as G1) or polypeptides of this invention. In one embodiment, a fusion polypeptide is provided that comprises at least 10 contiguous amino acids of the variable light chain region shown in SEQ ID NO:2 (Figure 5) and/or at least 10 amino acids of the variable heavy chain region shown in SEQ ID NO:1 (Figure 5). In other embodiments, a fusion polypeptide is provided that comprises at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable light chain region shown in SEQ ID NO:2 (Figure 5) and/or at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable heavy chain region shown in SEQ ID NO:1 (Figure 5). In another embodiment, the fusion polypeptide comprises a light chain variable region and/or a heavy chain variable region of G1, as shown in SEQ ID NO:2 and SEQ ID NO:1 of Figure 5. In another embodiment, the fusion polypeptide comprises one or more CDR(s) of G1. In still other embodiments, the fusion polypeptide comprises CDR H3 and/or CDR L3 of antibody G1. For purposes of this invention, an G1 fusion protein contains one or more G1 antibodies and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region. Exemplary heterologous sequences include, but are not limited to a "tag" such as a FLAG tag or a 6His tag (SEQ ID NO:56). Tags are well known in the art.

A G1 fusion polypeptide can be created by methods known in the art, for example, synthetically or recombinantly. Typically, the G1 fusion proteins of this invention are made by preparing an expressing a polynucleotide encoding them using recombinant methods described herein, although they may also be prepared by other means known in the art, including, for example, chemical synthesis.

In some aspects, this invention also provides compositions comprising antibodies or polypeptides derived from G1 conjugated (for example, linked) to an agent that facilitate coupling to a solid support (such as biotin or avidin). For simplicity, reference will be made generally to G1 or antibodies with the understanding that these methods apply to any of the CGRP binding embodiments described herein. Conjugation generally refers to linking these components as described herein. The linking (which is generally fixing these components in proximate association at least for administration) can be achieved in any number of ways. For example, a direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (e.g., a halide) on the other.

An antibody or polypeptide may be linked to a labeling agent (alternatively termed “label”) such as a fluorescent molecule, a radioactive molecule or any others labels known in the art. Labels are known in the art which generally provide (either directly or indirectly) a signal.

In some embodiments, the invention also provides compositions (including pharmaceutical compositions) and kits comprising antibody G1, and/or any or all of the antibodies or polypeptides described herein.

In some embodiments, the invention also provides isolated polynucleotides encoding the antibodies and polypeptides of the invention (including an antibody comprising the polypeptide sequences of the light chain and heavy chain variable regions shown in Figure 5), and vectors and host cells comprising the polynucleotide.

In some embodiments, the invention provides polynucleotides (or compositions, including pharmaceutical compositions), comprising polynucleotides encoding any of the following: (a) antibody G1 or its variants shown in Table 6; (b) a fragment or a region of antibody G1 or its variants shown in Table 6; (c) a light chain of antibody G1 or its variants shown in Table 6; (d) a heavy chain of antibody G1 or its variants shown in Table 6; (e) one or more variable region(s) from a light chain and/or a heavy chain of

antibody G1 or its variants shown in Table 6; (f) one or more CDR(s) (one, two, three, four, five or six CDRs) of antibody G1 or its variants shown in Table 6; (g) CDR H3 from the heavy chain of antibody G1; (h) CDR L3 from the light chain of antibody G1 or its variants shown in Table 6; (i) three CDRs from the light chain of antibody G1 or its variants shown in Table 6; (j) three CDRs from the heavy chain of antibody G1 or its variants shown in Table 6; (k) three CDRs from the light chain and three CDRs from the heavy chain, of antibody G1 or its variants shown in Table 6; and (l) an antibody comprising any one of (b) through (k). In some embodiments, the polynucleotide comprises either or both of the polynucleotide(s) shown in SEQ ID NO:9 and SEQ ID NO:10.

In another aspect, the invention provides polynucleotides encoding any of the antibodies (including antibody fragments) and polypeptides described herein, such as antibodies and polypeptides having impaired effector function. Polynucleotides can be made by procedures known in the art.

In another aspect, the invention provides compositions (such as a pharmaceutical compositions) comprising any of the polynucleotides of the invention. In some embodiments, the composition comprises an expression vector comprising a polynucleotide encoding the G1 antibody as described herein. In other embodiments, the composition comprises an expression vector comprising a polynucleotide encoding any of the antibodies or polypeptides described herein. In still other embodiments, the composition comprises either or both of the polynucleotides shown in SEQ ID NO:9 and SEQ ID NO:10. Expression vectors, and administration of polynucleotide compositions are further described herein.

In another aspect, the invention provides a method of making any of the polynucleotides described herein.

Polynucleotides complementary to any such sequences are also encompassed by the present invention. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain

introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (i.e., an endogenous sequence that encodes an antibody or a portion thereof) or may comprise a variant of such a sequence. Polynucleotide variants contain one or more substitutions, additions, deletions and/or insertions such that the immunoreactivity of the encoded polypeptide is not diminished, relative to a native immunoreactive molecule. The effect on the immunoreactivity of the encoded polypeptide may generally be assessed as described herein. Variants preferably exhibit at least about 70% identity, more preferably at least about 80% identity and most preferably at least about 90% identity to a polynucleotide sequence that encodes a native antibody or a portion thereof.

Two polynucleotide or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins - Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J., 1990, Unified Approach to Alignment and Phylogenes pp. 626-645 Methods in Enzymology vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M., 1989,

CABIOS 5:151-153; Myers, E.W. and Muller W., 1988, CABIOS 4:11-17; Robinson, E.D., 1971, Comb. Theor. 11:105; Santou, N., Nes, M., 1987, Mol. Biol. Evol. 4:406-425; Sneath, P.H.A. and Sokal, R.R., 1973, Numerical Taxonomy the Principles and Practice of Numerical Taxonomy, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J., 1983, Proc. Natl. Acad. Sci. USA 80:726-730.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the comparison window may comprise additions or deletions (i.e., gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (i.e., the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Variants may also, or alternatively, be substantially homologous to a native gene, or a portion or complement thereof. Such polynucleotide variants are capable of hybridizing under moderately stringent conditions to a naturally occurring DNA sequence encoding a native antibody (or a complementary sequence).

Suitable “moderately stringent conditions” include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X, and 0.2X SSC containing 0. 1 % SDS.

As used herein, "highly stringent conditions" or "high stringency conditions" are those that: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at 50°C; (2) employ during hybridization a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1% polyvinylpyrrolidone/50 mM sodium phosphate buffer at pH 6.5 with 750 mM sodium

chloride, 75 mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1% SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

It will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

The polynucleotides of this invention can be obtained using chemical synthesis, recombinant methods, or PCR. Methods of chemical polynucleotide synthesis are well known in the art and need not be described in detail herein. One of skill in the art can use the sequences provided herein and a commercial DNA synthesizer to produce a desired DNA sequence.

For preparing polynucleotides using recombinant methods, a polynucleotide comprising a desired sequence can be inserted into a suitable vector, and the vector in turn can be introduced into a suitable host cell for replication and amplification, as further discussed herein. Polynucleotides may be inserted into host cells by any means known in the art. Cells are transformed by introducing an exogenous polynucleotide by

direct uptake, endocytosis, transfection, F-mating or electroporation. Once introduced, the exogenous polynucleotide can be maintained within the cell as a non-integrated vector (such as a plasmid) or integrated into the host cell genome. The polynucleotide so amplified can be isolated from the host cell by methods well known within the art. See, e.g., Sambrook et al. (1989).

Alternatively, PCR allows reproduction of DNA sequences. PCR technology is well known in the art and is described in U.S. Patent Nos. 4,683,195, 4,800,159, 4,754,065 and 4,683,202, as well as PCR: The Polymerase Chain Reaction, Mullis et al. eds., Birkauswer Press, Boston (1994).

RNA can be obtained by using the isolated DNA in an appropriate vector and inserting it into a suitable host cell. When the cell replicates and the DNA is transcribed into RNA, the RNA can then be isolated using methods well known to those of skill in the art, as set forth in Sambrook et al., (1989), for example.

Suitable cloning vectors may be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors will generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, e.g., pUC18, pUC19, Bluescript (e.g., pBS SK+) and its derivatives, mp18, mp19, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

Expression vectors generally are replicable polynucleotide constructs that contain a polynucleotide according to any of the various aspects of the invention. It is implied that an expression vector must be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to,

one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (i.e., translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

The vectors containing the polynucleotides of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (e.g., where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell.

In some aspects, the invention also provides host cells comprising any of the polynucleotides described herein. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as E. coli or B. subtillis) and yeast (such as S. cerevisae, S. pombe; or K. lactis). Preferably, the host cells express the cDNAs at a level of about 5 fold higher, more preferably 10 fold higher, even more preferably 20 fold higher than that of the corresponding endogenous antibody or protein of interest, if present, in the host cells. Screening the host cells for a specific binding to Aϑ1-40 is effected by an immunoassay or FACS. A cell overexpressing the antibody or protein of interest can be identified.

D. Compositions

In some embodiments, compositions used in a method of the invention comprise an effective amount of an antibody (e.g., anti-CGRP antagonist antibody, monoclonal antibody that modulates the CGRP pathway) or an antibody derived polypeptide described herein. Examples of such compositions, as well as how to formulate, are also described in an earlier section and below. In one embodiment, the composition further

comprises a CGRP antagonist. In some embodiments, the composition comprises one or more monoclonal antibodies that modulate the CGRP pathway. In some embodiments, the composition comprises one or more anti-CGRP antagonist antibodies. In some embodiments, the anti-CGRP antagonist antibody recognizes human CGRP. In some embodiments, the anti-CGRP antagonist antibody is humanized. In some embodiments, the anti-CGRP antagonist antibody comprises a constant region that does not trigger an unwanted or undesirable immune response, such as antibody-mediated lysis or ADCC. In some embodiments, the anti-CGRP antagonist antibody comprises one or more CDR(s) of antibody G1 (such as one, two, three, four, five, or, in some embodiments, all six CDRs from G1). In some embodiments, the anti-CGRP antagonist antibody is human.

It is understood that the compositions can comprise more than one antibody (e.g., more than one anti-CGRP antagonist antibody -- a mixture of anti-CGRP antagonist antibodies that recognize different epitopes of CGRP). Other exemplary compositions comprise more than one anti-CGRP antagonist antibodies that recognize the same epitope(s), or different species of anti-CGRP antagonist antibodies that bind to different epitopes of CGRP.

A composition can further comprise pharmaceutically acceptable carriers, excipients, or stabilizers (Remington: The Science and practice of Pharmacy 20th Ed. (2000) Lippincott Williams and Wilkins, Ed. K. E. Hoover). Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed. A therapeutic formulation of an antibody may comprise one or more pharmaceutically acceptable carriers, excipients or stabilizes with non-limiting examples of such species that include buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic

polymers such as polyvinylpyrrolidone; amino acids (e.g., at concentrations of 0.1 mM to 100 mM, 0.1 mM to 1 mM, 0.01 mM to 50 mM, 1 mM to 50 mM, 1 mM to 30 mM, 1 mM to 20 mM, 10 mM to 25 mM) such as glycine, glutamine, methionine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents (e.g., at concentrations of 0.001 mg/mL to 1 mg/mL, 0.001 mg/mL to 1 mg/mL, 0.001 mg/mL to 0.1 mg/mL, 0.001 mg/mL to 0.01 mg/mL) such as EDTA (e.g., disodium EDTA dihydrate); sugars (e.g., at concentrations of 1 mg/mL to 500 mg/mL, 10 mg/mL to 200 mg/mL, 10 mg/mL to 100 mg/mL, 50 mg/mL to 150 mg/mL) such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants (e.g., at concentrations of 0.01 mg/mL to 10 mg/mL, 0.01 mg/mL to 1 mg/mL, 0.1 mg/mL to 1 mg/mL, 0.01 mg/mL to 0.5 mg/mL) such as TWEEN™ (e.g., polysorbate (e.g., polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80)), PLURONICS™ or polyethylene glycol (PEG). Pharmaceutically acceptable excipients are further described herein.

An antibody (e.g., an anti-CGRP antagonist antibody) and compositions thereof can also be used in conjunction with other agents that serve to enhance and/or complement the effectiveness of the agents.

E. Kits

In one aspect, the invention also provides kits for use in the instant methods. Kits can include one or more containers comprising an antibody described herein (e.g., an anti-CGRP antagonist antibody (such as a humanized antibody)) or polypeptide described herein and instructions for use in accordance with any of the methods described herein. Generally, these instructions comprise a description of administration of the antibody to treat, ameliorate or prevent post-traumatic headache according to any of the methods described herein. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has post-traumatic headache or whether the individual is at risk of having post-traumatic headache. In still other embodiments, the instructions comprise a description of

administering an antibody (e.g., anti-CGRP antagonist antibody) to an individual at risk of having post-traumatic headache.

In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is human. In other embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody comprises one or more CDR(s) of antibody G1 (such as one, two, three, four, five, or, in some embodiments, all six CDRs from G1).

The instructions relating to the use of an antibody (e.g., anti-CGRP antagonist antibody) generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (e.g., multi-dose packages) or sub-unit doses. Instructions supplied in the kits are typically written instructions on a label or package insert (e.g., a paper sheet included in the kit), but machine-readable instructions (e.g., instructions carried on a magnetic or optical storage disk) are also acceptable.

The label or package insert indicates that the composition is used for treating, ameliorating and/or preventing post-traumatic headache. Instructions may be provided for practicing any of the methods described herein.

The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (e.g., sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (e.g., an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-CGRP antagonist antibody and/or a monoclonal antibody that modulates the CGRP pathway. The container may further comprise a second pharmaceutically active agent.

Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or associated with the container.

The following Examples are provided to illustrate but not limit the invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application were specifically and individually indicated to be so incorporated by reference.

Further aspects and embodiments of the present invention are set out in the following numbered paragraphs:

1. A method of preventing, treating, or reducing incidence of post-traumatic headache in a subject comprising administering to the subject a monoclonal anti-CGRP antagonist antibody.
2. A method of preventing, treating, or reducing incidence of at least one secondary symptom associated with post-traumatic headache in a subject comprising administering to the subject a monoclonal anti-CGRP antagonist antibody.
3. The method of paragraph 1 or 2, wherein the monoclonal antibody is administered prior to, during and/or after post-traumatic headache.
4. The method of paragraph 3, wherein the monoclonal antibody is administered prior to the attack of post-traumatic headache (e.g., after trauma or injury to the head and/or neck).
5. The method of paragraph 1 or 2, wherein the monoclonal antibody is administered immediately after the trauma or injury to the head and/or neck.
6. The method of paragraph 1 or 2, wherein the amount of the monoclonal antibody administered to the subject is about 225 mg to about 1000 mg.

7. The method of paragraph 1 or 2, wherein the monoclonal antibody is administered subcutaneous.
8. The method of paragraph 1 or 2, wherein the monoclonal antibody is administered intravenous.
9. The method of paragraph 1 or 2, wherein the monoclonal antibody administered to the subject in an initial dose (e.g., a loading dose) and one or more additional doses (e.g., maintenance dose).
10. The method of paragraph 9, wherein the amount of the initial dose (e.g., a loading dose) and one or more additional doses (e.g., maintenance dose) is the same or different.
11. The method of paragraph 1 or 2, wherein the initial dose (e.g., a loading dose) and one or more additional doses (e.g., maintenance dose) are administered the same way, e.g., subcutaneously or intravenously.
12. The method of paragraph 1 or 2, wherein the one or more additional doses are administered in a different way than the initial dose (e.g., a loading dose), e.g., the initial dose is administered intravenously and the one or more additional doses (e.g., maintenance dose) is administered subcutaneously.
13. The method of paragraph 1 or 2, wherein the monoclonal antibody is administered monthly, quarterly, or a single dose.
14. The method of paragraph 1 or 2, wherein the monoclonal antibody is administered monthly.
15. The method of paragraph 1 or 2, wherein the monoclonal antibody is administered monthly for three or more months.
16. The method of paragraph 1or 2, wherein the dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months.
17. The method of paragraph 1 or 2, wherein the dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over

about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for about one year, e.g., two years, three years, four years, or five years.

18. The method of paragraph 1or 2, wherein the treating or reducing can comprise reducing the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing a 6-item Headache Impact Test (HIT-6) disability score, improving 12-Item Short Form Health Survey (SF-12) score (Ware et al., Med Care 4:220–233, 1996), reducing Patient Global Impression of Change (PGIC) score (Hurst et al., J Manipulative Physiol Ther 27:26-35, 2004), improving Sport ConCuSSion ASSeSment tool 3 (SCAT-3) score (McCrory et al. British Journal of Sports Medicine 47:263–266, 2013), or any combination thereof.

19. The method of paragraph 18, wherein the monthly headache hours experienced by the subject after said administering is reduced by 40 or more hours (e.g., 45, 50, 55, 60, 65, 70, 75, 80, or more) from a pre-administration level in the subject.

20. The method of paragraph 18, wherein the monthly headache hours experienced by the subject after said administering are reduced by 25% or more (e.g., 30%, 35%, 40%, 45%, 50%, or more) relative to a pre-administration level in the subject.

21. The method of paragraph 18, wherein the monthly headache days experienced by the subject after said administering is reduced by three or more days (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more days) from a pre-administration level in the subject.

22. The method of paragraph 1, wherein the administering can comprise utilizing a pre-filled syringe, pre-filled syringe with a needle safety device, injection pen, or auto-injector comprising a dose of the monoclonal antibody.

23. The method of paragraph 1, wherein the monoclonal antibody is formulated at a concentration of at least 150 mg/mL.

24. The method of paragraph 1, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL.

25. The method of paragraph 1, wherein the method further comprises administering to the subject a second agent simultaneously or sequentially with the monoclonal antibody.
26. The method of paragraph 1, wherein the second agent is any of 5-HT1 agonists, triptans, ergot alkaloids, and non-steroidal anti-inflammatory drugs. In some embodiments, the second agent is an agent taken by the subject prophylactically.
27. The method of paragraph 1, wherein the monthly use of the second agent by the subject is decreased by at least 15% after administering the monoclonal antibody.
28. The method of paragraph 1, wherein the second agent is a triptan.
29. The method of paragraph 1, wherein the subject is a human.
30. The method of paragraph 1, wherein the monoclonal antibody is a human or humanized monoclonal antibody.
31. The method of paragraph 1, wherein the monoclonal antibody is a humanized monoclonal antibody.
32. The method of paragraph 1, wherein the monoclonal antibody comprises a heavy chain constant region of the antibodies such as IgG1, IgG2, IgG3, and IgG4.
33. The method of paragraph 1, wherein the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.
34. The method of paragraph 1 or 2, wherein the post-traumatic headache is acute or persistent.
35. The method of paragraph 1 or 2, wherein the post-traumatic headache is persistent.
36. A composition for use in accordance with any of the preceding paragraphs.

1A. A method of preventing, treating, or reducing incidence of post-traumatic headache in a subject comprising administering to the subject a therapeutically effective amount of a monoclonal anti-CGRP antagonist antibody, wherein the monoclonal antibody is administered intravenously or subcutaneously at an initial loading dose of about 675 mg to about 900 mg followed by a subsequent maintenance dose of about 225 mg administered subcutaneously at one month intervals.

2A. A method of preventing, treating, or reducing incidence of at least one secondary symptom associated with post-traumatic headache in a subject comprising administering to the subject a therapeutically effective amount of a monoclonal anti-CGRP antagonist antibody, wherein the monoclonal antibody is administered intravenously at an initial loading dose of about 675 mg to about 900 mg followed by a subsequent maintenance dose of about 225 mg administered subcutaneously at one month intervals.

3A. The method of paragraph 1A or 2A, wherein the monoclonal antibody is administered prior to, during and/or after post-traumatic headache.

4A. The method of paragraph 3A, wherein the monoclonal antibody is administered prior to the attack of post-traumatic headache (e.g., after trauma or injury to the head and/or neck).

5A. The method of paragraph 1A or 2A, wherein the monoclonal antibody is administered immediately after the trauma or injury to the head and/or neck.

6A. The method of paragraph 1A or 2A, wherein the dosing regimen comprises administering an initial antibody dose of about 675 mg subcutaneously, followed by a monthly antibody dose of about 225 mg subcutaneously for about two months, e.g., about three months, four months, five months, six months, or 12 months.

7A. The method of paragraph 1A or 2A, wherein the treating or reducing can comprise reducing the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute

headache medications, reducing a 6-item Headache Impact Test (HIT-6) disability score, improving 12-Item Short Form Health Survey (SF-12) score (Ware et al., Med Care 4:220–233, 1996), reducing Patient Global Impression of Change (PGIC) score (Hurst et al., J Manipulative Physiol Ther 27:26-35, 2004), improving Sport ConCuSSion ASSeSment tool 3 (SCAT-3) score (McCrory et al. British Journal of Sports Medicine 47:263–266, 2013), or any combination thereof.

8A. The method of paragraph 1A or 2A, wherein the monthly headache hours experienced by the subject after said administering is reduced by 40 or more hours (e.g., 45, 50, 55, 60, 65, 70, 75, 80, or more) from a pre-administration level in the subject.

9A. The method of paragraph 1A or 2A, wherein the monthly headache hours experienced by the subject after said administering are reduced by 25% or more (e.g., 30%, 35%, 40%, 45%, 50%, or more) relative to a pre-administration level in the subject.

10A. The method of paragraph 1A or 2A, wherein the monthly headache days experienced by the subject after said administering is reduced by three or more days (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more days) from a pre-administration level in the subject.

11A. The method of paragraph 1A, wherein the administering can comprise utilizing a pre-filled syringe, pre-filled syringe with a needle safety device, injection pen, or auto-injector comprising a dose of the monoclonal antibody.

12A. The method of paragraph 1A, wherein the monoclonal antibody is formulated at a concentration of at least 150 mg/mL.

13A. The method of paragraph 1A, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL.

14A. The method of paragraph 1A, wherein the method further comprises administering to the subject a second agent simultaneously or sequentially with the monoclonal antibody.

15A. The method of paragraph 1A, wherein the second agent is any of 5-HT1 agonists, triptans, ergot alkaloids, and non-steroidal anti-inflammatory drugs. In some embodiments, the second agent is an agent taken by the subject prophylactically.

16A. The method of paragraph 1A, wherein the monthly use of the second agent by the subject is decreased by at least 15% after administering the monoclonal antibody.

17A. The method of paragraph 1A, wherein the second agent is a triptan.

18A. The method of paragraph 1A, wherein the subject is a human.

19A. The method of paragraph 1A, wherein the monoclonal antibody is a human or humanized monoclonal antibody.

20A. The method of paragraph 1A, wherein the monoclonal antibody is a humanized monoclonal antibody.

21A. The method of paragraph 1A, wherein the monoclonal antibody comprises a heavy chain constant region of the antibodies such as IgG1, IgG2, IgG3, and IgG4.

22A. The method of paragraph 1A, wherein the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

23A. The method of paragraph 1A or 2A, wherein the post-traumatic headache is acute or persistent.

24A. The method of paragraph 1A or 2A, wherein the post-traumatic headache is persistent.

25A. A composition for use in accordance with any of the preceding paragraphs.

1B. A method of preventing, treating, or reducing incidence of post-traumatic headache in a subject comprising administering to the subject a therapeutically effective amount of a monoclonal anti-CGRP antagonist antibody, wherein the monoclonal antibody is administered intravenously at a dose of about 675 mg to about 900 mg once per quarter.

2B. A method of preventing, treating, or reducing incidence of at least one secondary symptom associated with post-traumatic headache in a subject comprising administering to the subject a therapeutically effective amount of a monoclonal anti-CGRP antagonist antibody, wherein the monoclonal antibody is administered intravenously at an initial loading dose of about 675 mg to about 900 mg once per quarter.

3B. The method of paragraph 1B or 2B, wherein the monoclonal antibody is administered prior to, during and/or after post-traumatic headache.

4B. The method of paragraph 3B, wherein the monoclonal antibody is administered prior to the attack of post-traumatic headache (e.g., after trauma or injury to the head and/or neck).

5B. The method of paragraph 1B or 2B, wherein the monoclonal antibody is administered immediately after the trauma or injury to the head and/or neck.

6B. The method of paragraph 1B or 2B, wherein the dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every quarter for about one year, e.g., two years, three years, four years, or five years.

7B. The method of paragraph 1B or 2B, wherein the treating or reducing can comprise reducing the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing a 6-item Headache Impact Test (HIT-6) disability score, improving 12-Item Short Form Health Survey (SF-12) score (Ware et al., Med Care 4:220–233, 1996), reducing Patient Global Impression of

Change (PGIC) score (Hurst et al., J Manipulative Physiol Ther 27:26-35, 2004), improving Sport ConCuSSion ASSeSment tool 3 (SCAT-3) score (McCrory et al. British Journal of Sports Medicine 47:263–266, 2013), or any combination thereof.

8B. The method of paragraph 1B or 2B, wherein the monthly headache hours experienced by the subject after said administering is reduced by 40 or more hours (e.g., 45, 50, 55, 60, 65, 70, 75, 80, or more) from a pre-administration level in the subject.

9B. The method of paragraph 1B or 2B, wherein the monthly headache hours experienced by the subject after said administering are reduced by 25% or more (e.g., 30%, 35%, 40%, 45%, 50%, or more) relative to a pre-administration level in the subject.

10B. The method of paragraph 1B or 2B, wherein the monthly headache days experienced by the subject after said administering is reduced by three or more days (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more days) from a pre-administration level in the subject.

11B. The method of paragraph 1B, wherein the administering can comprise utilizing a pre-filled syringe, pre-filled syringe with a needle safety device, injection pen, or auto-injector comprising a dose of the monoclonal antibody.

12B. The method of paragraph 1B, wherein the monoclonal antibody is formulated at a concentration of at least 150 mg/mL.

13B. The method of paragraph 1B, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL.

14B. The method of paragraph 1B, wherein the method further comprises administering to the subject a second agent simultaneously or sequentially with the monoclonal antibody.

15B. The method of paragraph 1B, wherein the second agent is any of 5-HT1 agonists, triptans, ergot alkaloids, and non-steroidal anti-inflammatory drugs. In some embodiments, the second agent is an agent taken by the subject prophylactically.

16B. The method of paragraph 1B, wherein the monthly use of the second agent by the subject is decreased by at least 15% after administering the monoclonal antibody.

17B. The method of paragraph 1B, wherein the second agent is a triptan.

18B. The method of paragraph 1B, wherein the subject is a human.

19B. The method of paragraph 1B, wherein the monoclonal antibody is a human or humanized monoclonal antibody.

20B. The method of paragraph 1B, wherein the monoclonal antibody is a humanized monoclonal antibody.

21B. The method of paragraph 1B, wherein the monoclonal antibody comprises a heavy chain constant region of the antibodies such as IgG1, IgG2, IgG3, and IgG4.

22B. The method of paragraph 1B, wherein the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

23B. The method of paragraph 1B or 2B, wherein the post-traumatic headache is acute or persistent.

24B. The method of paragraph 1B or 2B, wherein the post-traumatic headache is persistent.

25B. A composition for use in accordance with any of the preceding paragraphs.

1C. A method of preventing, treating, or reducing incidence of post-traumatic headache in a subject comprising administering to the subject a therapeutically effective amount of a monoclonal anti-CGRP antagonist antibody, wherein the monoclonal antibody is administered intravenously at a dose of about 675 mg to about 900 mg once every three months intervals.

2C. A method of preventing, treating, or reducing incidence of at least one secondary symptom associated with post-traumatic headache in a subject comprising administering to the subject a therapeutically effective amount of a monoclonal anti-CGRP antagonist antibody, wherein the monoclonal antibody is administered intravenously at an initial loading dose of about 675 mg to about 900 mg once every three months intervals.

3C. The method of paragraph 1C or 2C, wherein the monoclonal antibody is administered prior to, during and/or after post-traumatic headache.

4C. The method of paragraph 3C, wherein the monoclonal antibody is administered prior to the attack of post-traumatic headache (e.g., after trauma or injury to the head and/or neck).

5C. The method of paragraph 1C or 2C, wherein the monoclonal antibody is administered immediately after the trauma or injury to the head and/or neck.

6C. The method of paragraph 1C or 2C, wherein the dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every three months for about one year, e.g., two years, three years, four years, or five years.

7C. The method of paragraph 1C or 2C, wherein the treating or reducing can comprise reducing the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing a 6-item Headache Impact Test (HIT-6) disability score, improving 12-Item Short Form Health Survey (SF-12) score (Ware et al., Med Care 4:220–233, 1996), reducing Patient Global Impression of

Change (PGIC) score (Hurst et al., J Manipulative Physiol Ther 27:26-35, 2004), improving Sport ConCuSSion ASSeSment tool 3 (SCAT-3) score (McCrory et al. British Journal of Sports Medicine 47:263–266, 2013), or any combination thereof.

8C. The method of paragraph 1C or 2C, wherein the monthly headache hours experienced by the subject after said administering is reduced by 40 or more hours (e.g., 45, 50, 55, 60, 65, 70, 75, 80, or more) from a pre-administration level in the subject.

9C. The method of paragraph 1C or 2C, wherein the monthly headache hours experienced by the subject after said administering are reduced by 25% or more (e.g., 30%, 35%, 40%, 45%, 50%, or more) relative to a pre-administration level in the subject.

10C. The method of paragraph 1C or 2C, wherein the monthly headache days experienced by the subject after said administering is reduced by three or more days (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more days) from a pre-administration level in the subject.

11C. The method of paragraph 1C, wherein the administering can comprise utilizing a pre-filled syringe, pre-filled syringe with a needle safety device, injection pen, or auto-injector comprising a dose of the monoclonal antibody.

12C. The method of paragraph 1C, wherein the monoclonal antibody is formulated at a concentration of at least 150 mg/mL.

13C. The method of paragraph 1C, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL.

14C. The method of paragraph 1C, wherein the method further comprises administering to the subject a second agent simultaneously or sequentially with the monoclonal antibody.

15C. The method of paragraph 1C, wherein the second agent is any of 5-HT1 agonists, triptans, ergot alkaloids, and non-steroidal anti-inflammatory drugs. In some embodiments, the second agent is an agent taken by the subject prophylactically.

16C. The method of paragraph 1C, wherein the monthly use of the second agent by the subject is decreased by at least 15% after administering the monoclonal antibody.

17C. The method of paragraph 1C, wherein the second agent is a triptan.

18C. The method of paragraph 1C, wherein the subject is a human.

19C. The method of paragraph 1C, wherein the monoclonal antibody is a human or humanized monoclonal antibody.

20C. The method of paragraph 1C, wherein the monoclonal antibody is a humanized monoclonal antibody.

21C. The method of paragraph 1C, wherein the monoclonal antibody comprises a heavy chain constant region of the antibodies such as IgG1, IgG2, IgG3, and IgG4.

22C. The method of paragraph 1C, wherein the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

23C. The method of paragraph 1C or 2C, wherein the post-traumatic headache is acute or persistent.

24C. The method of paragraph 1C or 2C, wherein the post-traumatic headache is persistent.

25C. A composition for use in accordance with any of the preceding paragraphs.

1D. A method of preventing, treating, or reducing incidence of post-traumatic headache in a subject comprising administering to the subject a therapeutically effective amount of a monoclonal anti-CGRP antagonist antibody, wherein the monoclonal antibody is administered intravenously at a dose of about 675 mg to about 900 mg once every six months intervals.

2D. A method of preventing, treating, or reducing incidence of at least one secondary symptom associated with post-traumatic headache in a subject comprising administering to the subject a therapeutically effective amount of a monoclonal anti-CGRP antagonist antibody, wherein the monoclonal antibody is administered intravenously at an initial loading dose of about 675 mg to about 900 mg once every six months intervals.

3D. The method of paragraph 1D or 2D, wherein the monoclonal antibody is administered prior to, during and/or after post-traumatic headache.

4D. The method of paragraph 3D, wherein the monoclonal antibody is administered prior to the attack of post-traumatic headache (e.g., after trauma or injury to the head and/or neck).

5D. The method of paragraph 1D or 2D, wherein the monoclonal antibody is administered immediately after the trauma or injury to the head and/or neck.

6D. The method of paragraph 1D or 2D, wherein the dosing regimen comprises administering an initial dose of about 900 mg intravenously in an infusion over about 60 minutes, followed by doses of about 900 mg administered intravenously in an infusion over about 60 minutes every six months for about one year, e.g., two years, three years, four years, or five years.

7D. The method of paragraph 1D or 2D, wherein the treating or reducing can comprise reducing the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing a 6-item Headache Impact Test (HIT-6) disability score, improving 12-Item Short Form Health Survey (SF-12) score (Ware et al., Med Care 4:220–233, 1996), reducing Patient Global Impression of

Change (PGIC) score (Hurst et al., J Manipulative Physiol Ther 27:26-35, 2004), improving Sport ConCuSSion ASSeSment tool 3 (SCAT-3) score (McCrory et al. British Journal of Sports Medicine 47:263–266, 2013), or any combination thereof.

8D. The method of paragraph 1D or 2D, wherein the monthly headache hours experienced by the subject after said administering is reduced by 40 or more hours (e.g., 45, 50, 55, 60, 65, 70, 75, 80, or more) from a pre-administration level in the subject.

9D. The method of paragraph 1D or 2D, wherein the monthly headache hours experienced by the subject after said administering are reduced by 25% or more (e.g., 30%, 35%, 40%, 45%, 50%, or more) relative to a pre-administration level in the subject.

10D. The method of paragraph 1D or 2D, wherein the monthly headache days experienced by the subject after said administering is reduced by three or more days (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more days) from a pre-administration level in the subject.

11D. The method of paragraph 1D, wherein the administering can comprise utilizing a pre-filled syringe, pre-filled syringe with a needle safety device, injection pen, or auto-injector comprising a dose of the monoclonal antibody.

12D. The method of paragraph 1D, wherein the monoclonal antibody is formulated at a concentration of at least 150 mg/mL.

13D. The method of paragraph 1D, wherein the monoclonal antibody is administered in a volume of less than 2 mL, e.g., about 1.5 mL.

14D. The method of paragraph 1D, wherein the method further comprises administering to the subject a second agent simultaneously or sequentially with the monoclonal antibody.

15D. The method of paragraph 1D, wherein the second agent is any of 5-HT1 agonists, triptans, ergot alkaloids, and non-steroidal anti-inflammatory drugs. In some embodiments, the second agent is an agent taken by the subject prophylactically.

16D. The method of paragraph 1D, wherein the monthly use of the second agent by the subject is decreased by at least 15% after administering the monoclonal antibody.

17D. The method of paragraph 1D, wherein the second agent is a triptan.

18D. The method of paragraph 1D, wherein the subject is a human.

19D. The method of paragraph 1D, wherein the monoclonal antibody is a human or humanized monoclonal antibody.

20D. The method of paragraph 1D, wherein the monoclonal antibody is a humanized monoclonal antibody.

21D. The method of paragraph 1D, wherein the monoclonal antibody comprises a heavy chain constant region of the antibodies such as IgG1, IgG2, IgG3, and IgG4.

22D. The method of paragraph 1D, wherein the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

23D. The method of paragraph 1D or 2D, wherein the post-traumatic headache is acute or persistent.

24D. The method of paragraph 1D or 2D, wherein the post-traumatic headache is persistent.

25D. A composition for use in accordance with any of the preceding paragraphs.

Examples

Example 1: Generation and characterization of monoclonal antibodies directed against CGRP

Generation of anti-CGRP antibodies. To generate anti-CGRP antibodies that have cross-species reactivity for rat and human CGRP, mice were immunized with 25-100 µg of human α-CGRP or β-CGRP conjugated to KLH in adjuvant (50 µl per footpad, 100 µl total per mouse) at various intervals. Immunization was generally performed as described in Geerligs HJ et al., 1989, J. Immunol. Methods 124:95-102; Kenney JS et al., 1989, J. Immunol. Methods 121:157-166; and Wicher K et al., 1989, Int. Arch. Allergy Appl. Immunol. 89:128-135. Mice were first immunized with 50 µg of human α-CGRP or β-CGRP conjugated to KLH in CFA (complete Freund's adjuvant). After 21 days, mice were secondly immunized with 25 µg of human β-CGRP (for mice first immunized with human α-CGRP) or α-CGRP (for mice first immunized with human β-CGRP) conjugated to KLH in IFA (incomplete Freund's adjuvant). Twenty-three days later after the second immunization, third immunization was performed with 25 µg of rat α-CGRP conjugated to KLH in IFA. Ten days later, antibody titers were tested using ELISA. Forth immunization was performed with 25 µg of the peptide (rat α-CGRP-KLH) in IFA 34 days after the third immunization. Final booster was performed with 100 µg soluble peptide (rat α-CGRP) 32 days after the forth immunization.

Splenocytes were obtained from the immunized mouse and fused with NSO myeloma cells at a ratio of 10:1, with polyethylene glycol 1500. The hybrids were plated out into 96-well plates in DMEM containing 20% horse serum and 2-oxaloacetate/pyruvate/insulin (Sigma), and hypoxanthine/aminopterin/thymidine selection was begun. On day 8, 100 µl of DMEM containing 20% horse serum was added to all the wells. Supernatants of the hybrids were screened by using antibody capture immunoassay. Determination of antibody class was done with class-specific second antibodies.

A panel of monoclonal antibody-producing cell lines was selected based on their binding to human and rat CGRP for further characterization. These antibodies and characteristics are shown below in Tables 2 and 3.

Purification and Fab fragment preparation. Monoclonal antibodies selected for further characterization were purified from supernatants of hybridoma cultures using protein A affinity chromatography. The supernatants were equilibrated to pH 8. The supernatants were then loaded to the protein A column MabSelect (Amersham Biosciences # 17-5199-02) equilibrated with PBS to pH 8. The column was washed with 5 column volumes of PBS, pH 8. The antibodies were eluted with 50 mM citrate-phosphate buffer, pH 3. The eluted antibodies were neutralized with 1 M Phosphate Buffer, pH 8. The purified antibodies were dialyzed with PBS, pH 7.4. The antibody concentrations were determined by SDS-PAGE, using a murine monoclonal antibody standard curve.

Fabs were prepared by papain proteolysis of the full antibodies using Immunopure Fab kit (Pierce # 44885) and purified by flow through protein A chromatography following manufacturer instructions. Concentrations were determined by ELISA and/or SDS-PAGE electrophoresis using a standard Fab of known concentration (determined by amino acid analysis), and by A280 using 1OD=0.6 mg/ml (or theoretical equivalent based on the amino acid sequence).

Affinity determination of the Fabs. Affinities of the anti-CGRP monoclonal antibodies were determined at either 25°C or 37°C using the BIACORE3000™ surface plasmon resonance (SPR) system (Biacore, INC, Piscataway NJ) with the manufacture's own running buffer, HBS-EP (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v polysorbate P20). Affinity was determined by capturing N-terminally biotinylated CGRP peptides (custom ordered from GenScript Corporation, New Jersey or Global Peptide Services, Colorado) via pre-immobilized streptavidin on SA chip and measuring binding kinetics of antibody Fab titrated across the CGRP surface. Biotinylated CGRP was diluted into HBS-EP and injected over the chip at a concentration of less than 0.001 mg/ml. Using variable flow time across the individual chip channels, two ranges of antigen density were achieved: <50 response units (RU) for detailed kinetic studies and about 800 RU for concentration studies and screening. Two- or three-fold serial dilutions typically at concentrations spanning 1 μM - 0.1 nM (aimed at 0.1-10x estimated K_D) of purified Fab fragments were injected for 1 minute at

100 µL/min and dissociation times of 10 minutes were allowed. After each binding cycle, surfaces were regenerated with 25 mM NaOH in 25% v/v ethanol, which was tolerated over hundreds of cycles. Kinetic association rate (k_{on}) and dissociation rate (k_{off}) were obtained simultaneously by fitting the data to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110) using the BIAevaluation program. Global equilibrium dissociation constants (K_D) or "affinities" were calculated from the ratio $K_D = k_{off}/k_{on}$. Affinities of the murine Fab fragments are shown in Tables 2 and 3.

Epitope mapping of the murine anti-CGRP antibodies. To determine the epitope that anti-CGRP antibodies bind on human α-CGRP, binding affinities of the Fab fragments to various CGRP fragments were measured as described above by capturing N-terminally biotinylated CGRP fragments amino acids 19-37 and amino acids 25-37 on a SA sensor chip. Figure 1 shows their binding affinities measured at 25°C. As shown in Figure 1, all antibodies, except antibody 4901, bind to human α-CGRP fragments 19-37 and 25-37 with affinity similar to their binding affinity to full length human α-CGRP (1-37). Antibody 4901 binds to human α-CGRP fragment 25-37 with six-fold lower affinity than binding to full length human α-CGRP fragment, due mainly to a loss in off-rate. The data indicate that these anti-CGRP antibodies generally bind to the C-terminal end of CGRP.

Alanine scanning was performed to further characterize amino acids in human α-CGRP involved in binding of anti-CGRP antibodies. Different variants of human α-CGRP with single alanine substitutions were generated by peptide synthesis. Their amino acid sequences are shown in Table 4 along with all the other peptides used in the Biacore analysis. Affinities of Fab fragments of the anti-CGRP antibodies to these variants were determined using Biacore as described above. As shown in Figure 1, all 12 antibodies target a C-terminal epitope, with amino acid F37 being the most crucial residue. Mutation of F37 to alanine significantly lowered the affinity or even completely knocked out binding of the anti-CGRP antibodies to the peptide. The next most important amino acid residue is G33, however, only the high affinity antibodies (7E9, 8B6, 10A8, and 7D11) were affected by alanine replacement at this position. Amino

acid residue S34 also plays a significant, but lesser, role in the binding of these four high affinity antibodies.

Table 2. Characteristics of the anti-CGRP monoclonal antibodies' binding to human α-CGRP and their antagonist activity

Antibodies	K_D to human α-CGRP at 25°C (nM)	K_D to human α-CGRP at 37°C (nM)	Cell-based blocking human α-CGRP binding to its receptor at 25°C (measured by cAMP activation)	IC_{50} (nM binding sites) at 25°C (room temp.) measured in radioligand binding assay.
7E9	1.0	0.9	Yes	2.5
8B6	1.1	1.2	Yes	4.0
10A8	2.1	3.0	Yes	n.d.
7D11	4.4	5.4	Yes	n.d.
6H2	9.3	42	Yes	12.9
4901	61	139	Yes	58
14E10	80	179	Yes	n.d.
9B8	85	183	No	n.d.
13C2	94	379	No	n.d.
14A9	148	581	No	n.d.
6D5	210	647	No	n.d.
1C5	296	652	No	n.d.

Note: Antibody 4901 is commercially available (Sigma, Product No. C7113).
n.d. = not determined

Table 3. Characteristics of the anti-CGRP monoclonal antibodies' binding to rat α-CGRP and antagonist activity

Antibodies	K_D to rat α-CGRP at 37°C (nM)	Cell-based blocking of binding of rat α-CGRP to its receptor at 25°C (measured by cAMP activation)	In vivo blocking in saphenous nerve assay
4901	3.4	Yes	Yes
7E9	47	Yes	Yes
6H2	54	No	No
8B6	75	Yes	Yes
7D11	218	Yes	Yes
10A8	451	No	n.d.
9B8	876	No	n.d.
14E10	922	No	n.d.
13C2	> 1000	No	n.d.
14A9	> 1000	No	n.d.
6D5	> 1000	No	n.d.
1C5	> 1000	No	n.d.

"n.d." indicates no test was performed for the antibody.

Table 4. Amino acid sequences of human α-CGRP fragments (SEQ ID NOS:15-40) and related peptides (SEQ ID NOS:41-47). All peptides are C-terminally amidated except SEQ ID NOS:36-40. Residues in bold indicate point mutations.

CGRP	Amino acid sequence	SEQ ID NO
1-37 (WT)	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	15
8-37	VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	16
19-37	SGGVVKNNFVPTNVGSKAF	17
P29A (19-37)	SGGVVKNNFVATNVGSKAF	18
K35A (19-37)	SGGVVKNNFVPTNVGSAAF	19
K35E (19-37)	SGGVVKNNFVPTNVGSEAF	20
K35M (19-37)	SGGVVKNNFVPTNVGSMAF	21
K35Q (19-37)	SGGVVKNNFVPTNVGSQAF	22
F37A (19-37)	SGGVVKNNFVPTNVGSKAA	23
25-38A	NNFVPTNVGSKAFA	24
25-37	NNFVPTNVGSKAF	25
F27A (25-37)	NNAVPTNVGSKAF	26
V28A (25-37)	NNFAPTNVGSKAF	27
P29A (25-37)	NNFVATNVGSKAF	28
T30A (25-37)	NNFVPANVGSKAF	29
N31A (25-37)	NNFVPTAVGSKAF	30
V32A (25-37)	NNFVPTNAGSKAF	31
G33A (25-37)	NNFVPTNVASKAF	32
S34A (25-37)	NNFVPTNVGAKAF	33
F37A (25-37)	NNFVPTNVGSKAA	34
26-37	NFVPTNVGSKAF	35
19-37-COOH	SGGVVKNNFVPTNVGSKAF	36
19-36-COOH	SGGVVKNNFVPTNVGSKA	37
1-36-COOH	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKA	38
1-19-COOH	ACDTATCVTHRLAGLLSRS	39
1-13-COOH	ACDTATCVTHRLA	40
rat α (1-37)	SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSEAF	41
rat α (19-37)	SGGVVKDNFVPTNVGSEAF	42
human β (1-37)	ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF	43
rat β (1-37)	SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSKAF	44
Human calcitonin (1-32)	CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP	45
Human amylin (1-37)	KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY	46
Human adrenomedullin (1-52)	YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDK DKDNVAPRSKISPQGY	47

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Example 2: Screening of anti-CGRP antagonist antibodies using in vitro assays.

Murine anti-CGRP antibodies were further screened for antagonist activity in vitro using cell based cAMP activation assay and binding assay.

Antagonist activity measured by cAMP assay. Five microliters of human or rat α-CGRP (final concentration 50 nM) in the presence or absence of an anti-CGRP antibody (final concentration 1-3000 nM), or rat α-CGRP or human α-CGRP (final concentration 0.1 nM-10 μM; as a positive control for c-AMP activation) was dispensed into a 384-well plate (Nunc, Cat. No. 264657). Ten microliters of cells (human SK-N-MC if human α-CGRP is used, or rat L6 from ATCC if rat α-CGRP is used) in stimulation buffer (20 mM HEPES, pH 7.4, 146 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 500 μM 3-Isobutyl-1-methylxanthine (IBMX)) were added into the wells of the plate. The plate was incubated at room temperature for 30 minutes.

After the incubation, cAMP activation was performed using HitHunter™ Enzyme Fragment Complementation Assay (Applied Biosystems) following manufacture's instruction. The assay is based on a genetically engineered β-galactosidase enzyme that consists of two fragments -termed Enzyme Acceptor (EA) and Enzyme Donor (ED). When the two fragments are separated, the enzyme is inactive. When the fragments are together they can recombine spontaneously to form active enzyme by a process called complementation. The EFC assay platform utilizes an ED-cAMP peptide conjugate in which cAMP is recognized by anti-cAMP. This ED fragment is capable of reassociation with EA to form active enzyme. In the assay, anti-cAMP antibody is optimally titrated to bind ED-cAMP conjugate and inhibit enzyme formation. Levels of cAMP in cell lysate samples compete with ED-cAMP conjugate for binding to the anti-cAMP antibody. The amount of free ED conjugate in the assay is proportional to the concentration of cAMP. Therefore, cAMP is measured by the formation of active enzyme that is quantified by the turnover of β-galactosidase luminescent substrate. The cAMP activation assay was performed by adding 10 μl of lysis buffer and anti-cAMP antibody (1:1 ratio) following by incubation at room temperature for 60 min. Then 10 μl of ED-cAMP reagent was added into each well and incubated for 60 minutes at room temperature. After the incubation, 20 μl of EA reagent and CL mixture (containing the

substrate) (1:1 ratio) was added into each well and incubated for 1-3 hours or overnight at room temperature. The plate was read at 1 second/well on PMT instrument or 30 seconds/place on imager. The antibodies that inhibit activation of cAMP by α-CGRP were identified (referred to as "yes") in Tables 2 and 3 above. Data in Tables 2 and 3 indicate that antibodies that demonstrated antagonist activity in the assay generally have high affinity. For example, antibodies having K_D (determined at 25°C) of about 80 nM or less to human α-CGRP or having K_D (determined at 37°C) of about 47 nM or less to rat α-CGRP showed antagonist activity in this assay.

Radioligand binding assay. Binding assay was performed to measure the IC_{50} of anti-CGRP antibody in blocking the CGRP from binding to the receptor as described previously. Zimmermann et al., Peptides 16:421-4, 1995; Mallee et al., J. Biol. Chem. 277:14294-8, 2002. Membranes (25 μg) from SK-N-MC cells were incubated for 90 min at room temperature in incubation buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.1% BSA) containing 10 pM ^{125}I-human α-CGRP in a total volume of 1 mL. To determine inhibition concentrations (IC_{50}), antibodies or unlabeled CGRP (as a control), from a about 100 fold higher stock solution were dissolved at varying concentrations in the incubation buffer and incubated at the same time with membranes and 10 pM ^{125}I-human α-CGRP. Incubation was terminated by filtration through a glass microfiber filter (GF/B, 1 μm) which had been blocked with 0.5% polyethylemimine. Dose response curves were plotted and K_i values were determined by using the equation: $K_i = IC_{50}/(1+([ligand]/K_D)$; where the equilibrium dissociation constant K_D = 8 pM for human α-CGRP to CGRP1 receptor as present in SK-N-MC cells, and B_{max} = 0.025 pmol/mg protein. The reported IC_{50} value (in terms of IgG molecules) was converted to binding sites (by multiplying it by 2) so that it could be compared with the affinities (K_D) determined by Biacore (see Table 2).

Table 2 shows the IC_{50} of murine antibodies 7E9, 8B6, 6H2 and 4901. Data indicate that antibody affinity generally correlates with IC_{50}: antibodies with higher affinity (lower K_D values) have lower IC_{50} in the radioligand binding assay.

Example 3: Effect of anti-CGRP antagonist antibodies on skin vasodilatation induced by stimulation of rat saphenous nerve

To test antagonist activity of anti-CGRP antibodies, effect of the antibodies on skin vasodilatation by stimulation of rat saphenous nerve was tested using a rat model described previously. Escott et al., Br. J. Pharmacol. 110:772-776, 1993. In this rat model, electrical stimulation of saphenous nerve induces release of CGRP from nerve endings, resulting in an increase in skin blood flow. Blood flow in the foot skin of male Sprague Dawley rats (170-300 g, from Charles River Hollister) was measured after saphenous nerve stimulation. Rats were maintained under anesthesia with 2% isoflurane. Bretylium tosylate (30 mg/kg, administered i.v.) was given at the beginning of the experiment to minimize vasoconstriction due to the concomitant stimulation of sympathetic fibers of the saphenous nerve. Body temperature was maintained at 37°C by the use of a rectal probe thermostatically connected to a temperature controlled heating pad. Compounds including antibodies, positive control (CGRP 8-37), and vehicle (PBS, 0.01% Tween 20) were given intravenously through the right femoral vein, except for the experiment shown in Figure 3, the test compound and the control were injected through tail vein, and for experiments shown in Figures 2A and 2B, antibodies 4901 and 7D11 were injected intraperitoneally (IP). Positive control compound CGRP 8-37 (vasodilatation antagonist), due to its short half-life, was given 3-5 min before nerve stimulation at 400 nmol/kg (200 μl). Tan et al., Clin. Sci. 89:656-73, 1995. The antibodies were given in different doses (1 mg/kg, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, and 25 mg/kg).

For experiments shown in Figures 2A and 2B, antibody 4901 (25 mg/kg), antibody 7D11 (25 mg/kg), or vehicle control (PBS with 0.01% Tween 20) was administered intraperitoneally (IP) 72 hours before the electrical pulse stimulation. For experiment shown in Figure 3, antibody 4901 (1 mg/kg, 2.5 mg/kg, 5 mg/kg, or 25 mg/kg) or vehicle control (PBS with 0.01% Tween 20) was administered intravenously 24 hours before the electrical pulse stimulation. After administration of the antibodies or vehicle control, the saphenous nerve of the right hindlimb was exposed surgically, cut proximally and covered with plastic wrap to prevent drying. A

laser Doppler probe was placed over the medio-dorsal side of the hindpaw skin, which is the region innervated by the saphenous nerve. Skin blood flow, measured as blood cell flux, was monitored with a laser Doppler flow meter. When a stable base-line flux (less than 5% variation) was established for at least 5 minutes, the nerve was placed over platinum bipolar electrodes and electrically stimulated with 60 pulses (2 Hz, 10 V, 1 ms, for 30 seconds) and then again 20 minutes later. Cumulative change in skin blood flow was estimated by the area under the flux-time curve (AUC, which is equal to change in flux multiplied by change in time) for each flux response to electrical pulse stimulation. The average of the blood flow response to the two stimulations was taken. Animals were kept under anesthesia for a period of one to three hours.

As shown in Figure 2A and Figure 2B, blood flow increase stimulated by applying electronic pulses on saphenous nerve was inhibited by the presence of CGRP 8-37 (400 nmol/kg, administered i.v.), antibody 4901 (25 mg/kg, administered ip), or antibody 7D11 (25 mg/kg, administered ip) as compared to the control. CGRP 8-37 was administered 3-5 minutes before the saphenous nerve stimulation; and antibodies were administered 72 hours before the saphenous nerve stimulation. As shown in Figure 3, blood flow increase stimulated by applying electronic pulses on saphenous nerve was inhibited by the presence of antibody 4901 at different doses (1 mg/kg, 2.5 mg/kg, 5 mg/kg, and 25 mg/kg) administered intravenously at 24 hours before the saphenous nerve stimulation.

For experiments shown in Figures 4A and 4B, saphenous nerve was exposed surgically before antibody administration. The saphenous nerve of the right hindlimb was exposed surgically, cut proximally and covered with plastic wrap to prevent drying. A laser Doppler probe was placed over the medio-dorsal side of the hindpaw skin, which is the region innervated by the saphenous nerve. Skin blood flow, measured as blood cell flux, was monitored with a laser Doppler flow meter. Thirty to forty-five minutes after bretylium tosylate injection, when a stable base-line flux (less than 5% variation) was established for at least 5 minutes, the nerve was placed over platinum bipolar electrodes and electrically stimulated (2Hz, 10V, 1 ms, for 30 sec) and again 20 minutes later. The average of the blood flow flux response to these two stimulations

was used to establish the baseline response (time 0) to electrical stimulation. Antibody 4901 (1 mg/kg or 10 mg/kg), antibody 7E9 (10 mg/kg), antibody 8B6 (10 mg/kg), or vehicle (PBS with 0.01% Tween 20) were then administered intravenously (i.v.). The nerve was subsequently stimulated (2Hz, 10V, 1 ms, for 30 sec) at 30 minutes, 60 minutes, 90 minutes, and 120 minutes after antibody or vehicle administration. Animals were kept under anesthesia for a period of approximately three hours. Cumulative change in skin blood flow was estimated by the area under the flux-time curve (AUC, which is equal to change in flux multiplied by change in time) for each flux response to electrical pulse stimulations.

As shown in Figure 4A, blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody 4901 1 mg/kg administered i.v., when electronic pulse stimulation was applied at 60 minutes, 90 minutes, and 120 minutes after the antibody administration, and blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody 4901 10 mg/kg administered i.v., when electronic pulse stimulation was applied at 30 minutes, 60 minutes, 90 minutes, and 120 minutes after the antibody administration. Figure 4B shows that blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody 7E9 (10 mg/kg, administered i.v.) when electronic pulse stimulation was applied at 30 min, 60 min, 90 min, and 120 min after antibody administration, and by the presence of antibody 8B6 (10 mg/kg, administered i.v.) when electronic pulse stimulation was applied at 30 min after antibody administration.

These data indicate that antibodies 4901, 7E9, 7D11, and 8B6 are effective in blocking CGRP activity as measured by skin vasodilatation induced by stimulation of rat saphenous nerve.

Example 4. Characterization of anti-CGRP antibody G1 and its variants

Amino acid sequences for the heavy chain variable region and light chain variable region of anti-CGRP antibody G1 are shown in Figure 5. The following methods were used for expression and characterization of antibody G1 and its variants.

Expression vector used. Expression of the Fab fragment of the antibodies was under control of an IPTG inducible lacZ promoter similar to that described in Barbas (2001) Phage display: a laboratory manual, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press pg. 2.10. Vector pComb3X), however, modifications included addition and expression of the following additional domains: the human Kappa light chain constant domain and the CH1 constant domain of IgG2 human immunoglobulin, Ig gamma-2 chain C region, protein accession number P01859; Immunoglobulin kappa light chain (Homo sapiens), protein accession number CAA09181.

Small scale Fab preparation. From E. coli transformed (either using electroporation-competent TG1 cells or chemically-competent Top 10 cells) with a Fab library, single colonies were used to inoculate both a master plate (agar LB + carbenicillin (50 µg/mL) + 2% glucose) and a working plate (2 mL/well, 96-well/plate) where each well contained 1.5 mL LB + carbenicillin (50 µg/mL) + 2% glucose. A gas permeable adhesive seal (ABgene, Surrey, UK) was applied to the plate. Both plates were incubated at 30°C for 12-16 hours; the working plate was shaken vigorously. The master plate was stored at 4°C until needed, while the cells from the working plate were pelleted (4000 rpm, 4°C, 20 minutes) and resuspended in 1.0 mL LB + carbenicillin (50 µg/mL) + 0.5 mM IPTG to induce expression of Fabs by vigorous shaking for 5 hours at 30°C. Induced cells were centrifuges at 4000 rpm, 4°C for 20 minutes and resuspended in 0.6 mL Biacore HB-SEP buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v P20). Lysis of HB-SEP resuspended cells was accomplished by freezing (-80°C) and then thawing at 37°C. Cell lysates were centrifuged at 4000 rpm, 4°C for 1 hour to separate the debris from the Fab-containing supernatants, which were subsequently filtered (0.2 µm) using a Millipore MultiScreen Assay System 96-Well Filtration Plate and vacuum manifold. Biacore was used to analyze filtered supernatants by injecting them across CGRPs on the sensor chip. Affinity-selected clones expressing Fabs were rescued from the master plate, which provided template DNA for PCR, sequencing, and plasmid preparation.

Large scale Fab preparation. To obtain kinetic parameters, Fabs were expressed on a larger scale as follows. Erlenmeyer flasks containing 150 mL LB +

carbenicillin (50 µg/mL) + 2% glucose were inoculated with 1 mL of a "starter" overnight culture from an affinity-selected Fab-expressing E. coli clone. The remainder of the starter culture (~3 mL) was used to prepare plasmid DNA (QIAprep mini-prep, Qiagen kit) for sequencing and further manipulation. The large culture was incubated at 30°C with vigorous shaking until an OD_{600nm} of 1.0 was attained (typically 12-16 h). The cells were pelleted by centrifuging at 4000 rpm, 4°C for 20 minutes, and resuspended in 150 mL LB + carbenicillin (50 µg/mL) + 0.5 mM IPTG. After 5 hours expression at 30°C, cells were pelleted by centrifuging at 4000 rpm, 4°C for 20 minutes, resuspended in 10 mL Biacore HBS-EP buffer, and lysed using a single freeze (-80°C)/thaw (37°C) cycle. Cell lysates were pelleted by centrifuging at 4000rpm, 4°C for one hour, and the supernatant was collected and filtered (0.2um). Filtered supernatants were loaded onto Ni-NTA superflow sepharose (Qiagen, Valencia, CA) columns equilibrated with PBS, pH 8, then washed with 5 column volumes of PBS, pH 8. Individual Fabs eluted in different fractions with PBS (pH 8) + 300 mM Imidazole. Fractions containing Fabs were pooled and dialyzed in PBS, then quantified by ELISA prior to affinity characterization.

Full antibody preparation. For expression of full antibodies, heavy and light chain variable regions were cloned in mammalian expression vectors and transfected using lipofectamine into HEK 293 cells for transient expression. Antibodies were purified using protein A using standard methods.

Vector pDb.CGRP.hFcGI is an expression vector comprising the heavy chain of the G1 antibody, and is suitable for transient or stable expression of the heavy chain. Vector pDb.CGRP.hFcGI has nucleotide sequences corresponding to the following regions: the murine cytomegalovirus promoter region (nucleotides 7-612); a synthetic intron (nucleotides 613-1679); the DHFR coding region (nucleotides 688-1253); human growth hormone signal peptide (nucleotides 1899-1976); heavy chain variable region of G1 (nucleotides 1977-2621); human heavy chain IgG2 constant region containing the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence; see Eur. J. Immunol. (1999) 29:2613-2624). Vector pDb.CGRP.hFcGI was deposited at the ATCC on July 15, 2005, and was assigned ATCC Accession No. PTA-6867.

Vector pEb.CGRP.hKGl is an expression vector comprising the light chain of the G1 antibody, and is suitable for transient expression of the light chain. Vector pEb.CGRP.hKGl has nucleotide sequences corresponding to the following regions: the murine cytomegalovirus promoter region (nucleotides 2-613); human EF-1 intron (nucleotides 614-1149); human growth hormone signal peptide (nucleotides 1160-1237); antibody G1 light chain variable region (nucleotides 1238-1558); human kappa chain constant region (nucleotides 1559-1882). Vector pEb.CGRP.hKGl was deposited at the ATCC on July 15, 2005, and was assigned ATCC Accession No. PTA-6866.

Biacore assay for affinity determination. Affinities of G1 monoclonal antibody and its variants were determined at either 25°C or 37°C using the BIACORE3000™ surface plasmon resonance (SPR) system (Biacore, INC, Piscataway NJ). Affinity was determined by capturing N-terminally biotinylated CGRP or fragments via pre-immobilized streptavidin (SA sensor chip) and measuring the binding kinetics of antibody G1 Fab fragments or variants titrated across the CGRP or fragment on the chip. All Biacore assays were conducted in HBS-EP running buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v polysorbate P20). CGRP surfaces were prepared by diluting the N-biotinylated CGRP to a concentration of less than 0.001 mg/mL into HBS-EP buffer and injecting it across the SA sensor chip using variable contact times. Low capacity surfaces, corresponding to capture levels <50 response units (RU) were used for high-resolution kinetic studies, whereas high capacity surfaces (about 800 RU of captured CGRP) were used for concentration studies, screening, and solution affinity determinations. Kinetic data were obtained by diluting antibody G1 Fab serially in two- or three-fold increments to concentrations spanning 1uM-0.1nM (aimed at 0.1-10x estimated K_D). Samples were typically injected for 1minute at 100 μL/min and dissociation times of at least 10 minutes were allowed. After each binding cycle, surfaces were regenerated with 25 mM NaOH in 25% v/v ethanol, which was tolerated over hundreds of cycles. An entire titration series (typically generated in duplicate) was fit globally to a 1:1 Langmuir binding model using the BIAevaluation program. This returned a unique pair of association and dissociation kinetic rate constants (respectively, k_{on} and k_{off}) for each binding interaction, whose ratio

gave the equilibrium dissociation constant (K_D = k_{off}/k_{on}). Affinities (K_D values) determined in this way are listed in Tables 6 and 7.

High-resolution analysis of binding interactions with extremely slow offrates. For interactions with extremely slow offrates (in particular, antibody G1 Fab binding to human α-CGRP on the chip at 25°C), affinities were obtained in a two-part experiment. The protocol described above was used with the following modifications. The association rate constant (k_{on}) was determined by injecting a 2-fold titration series (in duplicate) spanning 550 nM-1 nM for 30 seconds at 100 µL/min and allowing only a 30 second dissociation phase. The dissociation rate constant (k_{off}) was determined by injecting three concentrations (high, medium, and low) of the same titration series in duplicate for 30 seconds and allowing a 2-hour dissociation phase. The affinity (K_D) of each interaction was obtained by combining the k_{on} and k_{off} values obtained in both types of experiments, as shown in Table 5.

Determining solution affinity by Biacore. The solution affinity of antibody G1 for rat α-CGRP and F37A (19-37) human α-CGRP was measured by Biacore at 37°C. A high capacity CGRP chip surface was used (the high-affinity human α-CGRP was chosen for detection purposes) and HBS-EP running buffer was flowed at 5 µL/min. Antibody G1 Fab fragment at a constant concentration of 5 nM (aimed to be at or below the expected K_D of the solution-based interaction) was pre-incubated with competing peptide, either rat α-CGRP or F37A (19-37) human α-CGRP, at final concentrations spanning 1 nM to 1 µM in 3-fold serial dilutions. Antibody G1 Fab solutions in the absence or presence of solution-based competing peptide, were injected across CGRP on the chip and the depletion of binding responses detected at the chip surface as a result of solution competition was monitored. These binding responses were converted to "free Fab concentrations" using a calibration curve, which was constructed by titrating antibody G1 Fab alone (5, 2.5, 1.25, 0.625, 0.325 and 0 nM) across the CGRP on the chip. "Free Fab concentrations" were plotted against the concentration of competing solution-based peptide used to generate each data point and fit to a solution affinity model using the BIAevaluation software. The solution affinities determined (indirectly) in this way are shown in Tables 5 and 7 and were used to validate the affinities obtained

when Fabs are injected directly across N-biotinylated CGRPs on a SA chip. The close agreement between the affinities determined by these two methods confirms that tethering an N-biotinylated version of the CGRP to the chip does not alter its native solution binding activity.

Table 5 below shows the binding affinities of antibody G1 to human α-CGRP, human β-CGRP, rat α-CGRP, and rat β-CGRP determined by Biacore, by flowing Fab fragments across N-biotinylated CGRPs on a SA chip. To better resolve the affinities of binding interactions with extremely slow offrates, affinities were also determined in a two-part experiment to complement this assay orientation, the solution affinity of the rat α-CGRP interaction was also determined (as described above). The close agreement of the affinities measured in both assay orientations confirms that the binding affinity of the native rat α-CGRP in solution is not altered when it is N-biotinylated and tethered to a SA chip.

Table 5. Binding affinities of antibody G1 Fabs titrated across CGRPs on the chip

CGRP on chip	Temp. (°C)	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
Human α-CGRP	25	1.86×10^5	7.80×10^{-6}	0.042 (7%, n=4)*
Human α-CGRP	37	5.78×10^5	3.63×10^{-5}	0.063 (4%, n=2)*
Human β-CGRP	37	4.51×10^5	6.98×10^{-5}	0.155
Rat α-CGRP	25	5.08×10^4	6.18×10^{-5}	1.22 (12%, n=2)*
Rat α-CGRP	37	1.55×10^5	3.99×10^{-4}	2.57* (Solution K_D=10 (50%, n=4)**
Rat β-CGRP	37	5.16×10^5	7.85×10^{-5}	0.152

*Affinities for α-CGRPs (rat and human) were determined in a high-resolution two-part experiment, in which the dissociation phase was monitored for 2 hours (the values for k_{on}, k_{off}, and K_D represent the average of n replicate experiments with the standard deviation expressed as a percent variance). Affinities for β-CGRPs (rat and human) were determined by global analysis using only a 20-min dissociation phase, which was not accurate enough to quantify their extremely offrates (their offrates are likely slower than stated here and therefore their affinities are likely even higher). Antibody G1 Fab dissociated extremely slowly from all CGRPs (except α-rat CGRP) with offrates that approached the resolution limit of the Biacore assay (especially at 25°C).

**Solution affinity determined by measuring the depletion of binding responses detected at CGRP on the chip for antibody G1 Fab pre-incubated with solution-based rat α-CGRP competitor.

Table 6 below shows antibodies having the amino acid sequence variation as compared to antibody G1 and their affinities to both rat α-CGRP and human α-CGRP. All amino acid substitutions of the variants shown in Table 6 are described relative to the sequence of G1. The binding affinities of Fab fragments were determined by Biacore by flowing them across CGRPs on a SA chip.

Table 6. Amino acid sequences and binding affinity data for antibody G1 variants determined at 37°C by Biacore.

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
G1					$3.99x10^{-4}$	2.57	$3.63 x10^{-5}$	0.063
M1				A100L	$1.10x10^{-3}$		$1.73x10^{-4}$	
M2				L99A A100R	$2.6x10^{-3}$	58	$3.1x10^{-4}$	3
M3				L99A A100S	$2.0x10^{-3}$	61	$2.1x10^{-4}$	1.7
M4				L99A A100V	$1.52x10^{-3}$	84.4	$6.95x10^{-5}$	0.43
M5				L99A A100Y	$7.35x10^{-4}$	40.8	$3.22x10^{-5}$	0.20
M6				L99N	$7.84x10^{-4}$	43.6	$1.33x10^{-4}$	0.83
M7				L99N A100C	$9.18x10^{-4}$	51.0	$2.43x10^{-4}$	1.52
M8				L99N A100G	$7.45x10^{-4}$	41.4	$9.20x10^{-5}$	0.58
M9				L99N A100Y	n.d.	n.d.	$1.00x10^{-5}$	0.06
M10				L99S A100S	$1.51x10^{-3}$	83.9	$1.73x10^{-4}$	1.08
M11				L99S A100T	$4.83x10^{-3}$	268.3	$2.83x10^{-4}$	1.77
M12				L99S A100V	$1.94x10^{-3}$	107.8	$1.01x10^{-4}$	0.63
M13				L99T A100G	$1.84x10^{-3}$	102.2	$1.86x10^{-4}$	1.16
M14				L99T A100K	n.d.	n.d.	$1.00x10^{-5}$	0.06
M15				L99T A100P	$1.15x10^{-3}$	63.9	$1.58x10^{-5}$	0.10

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
M16				L99T A100S	$9.96x10^{-4}$	55.3	$1.65x10^{-4}$	1.03
M17				L99T A100V	$2.06x10^{-3}$	114.4	$1.85x10^{-4}$	1.16
M18				L99V A100G	$1.22x10^{-3}$	67.8	$7.03x10^{-5}$	0.44
M19				L99V A100R	n.d.	n.d.	$1.00x10^{-5}$	0.06
M20	R28W			L99R A100L	$1.44x10^{-3}$	80.0	$1.36x10^{-4}$	0.85
M21	R28W			L99S	$6.95x10^{-4}$	15.2	$1.42x10^{-4}$	1.23
M22	R28W			L99T	$1.10x10^{-3}$	61.1	$1.16x10^{-4}$	0.73
M23	R28G			L99T A100V	$7.99x10^{-4}$	44.4	$1.30x10^{-4}$	0.81
M24	R28L			L99T A100V	$1.04x10^{-3}$	57.8	$1.48x10^{-4}$	0.93
M25	R28N			L99T A100V	$1.4x10^{-3}$	76	$1.4x10^{-4}$	1.3
M26	R28N		A57G	L99T A100V	$9.24x10^{-4}$	51.3	$1.48x10^{-4}$	0.93
M27	R28N T30A			L99T A100V	$3.41x10^{-3}$	189.4	$3.57x10^{-4}$	2.23
M28	R28N T30D		E54R A57N	L99T A100V	$1.25x10^{-3}$	69.4	$9.96x10^{-5}$	0.62
M29	R28N T30G			L99T A100V	$3.59x10^{-3}$	199.4	$3.80x10^{-4}$	2.38
M30	R28N T30G		E54K A57E	L99T A100V	$6.38x10^{-3}$	354.4	$5.90x10^{-4}$	3.69
M31	R28N T30G		E54K A57G	L99T A100V	$3.61x10^{-3}$	200.6	$3.47x10^{-4}$	2.17
M32	R28N T30G		E54K A57H	L99T A100V	$2.96x10^{-3}$	164.4	$2.71x10^{-4}$	1.69
M33	R28N T30G		E54K A57N S58G	L99T A100V	$9.22x10^{-3}$	512.2	$7.50x10^{-4}$	4.69
M34	R28N T30G		E54K A57N S58T	L99T A100V	$2.17x10^{-3}$	120.6	$6.46x10^{-4}$	4.04
M35	R28N T30G		E54K A57S	L99T A100V	$3.99x10^{-3}$	221.7	$3.39x10^{-4}$	2.12
M36	R28N T30R			L99T A100V	$4.79x10^{-3}$	266.1	$2.39x10^{-4}$	1.49
M37	R28N T30S		A57G	L99T A100V	$1.45x10^{-3}$	80.6	$2.26x10^{-4}$	1.41
M38	R28N T30W			L99T A100V	$5.11x10^{-3}$	283.9	$2.18x10^{-4}$	1.36
M39	R28N	G50A L56T	A57N S58Y	L99T A100V	$9.95x10^{-3}$	552.8	$4.25x10^{-4}$	2.66
M40	R28N	G50A L56T	E54K A57L	L99T A100V	0.36	20000.0	$1.28x10^{-3}$	8.00
M41	R28N	G50A	E54K	L99T	$4.53x10^{-3}$	251.7	$2.10x10^{-4}$	1.31

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
		L56T	A57N E64D	A100V				
M42	R28N	G50A L56T	E54K A57N H61F	L99T A100V	$7.52x10^{-3}$	417.8	$4.17x10^{-4}$	2.61
M43	R28N	G50A L56T	E54K A57N S58C	L99T A100V	$4.53x10^{-3}$	251.7	$2.63x10^{-4}$	1.64
M44	R28N	G50A L56T	E54K A57N S58E	L99T A100V	$6.13x10^{-3}$	443	$2.10x10^{-4}$	2.05
M45	R28N	G50A L56T	E54K A57N S58E E64D	L99T A100V	$5.58x10^{-3}$	259	$2.11x10^{-4}$	1.85
M46	R28N	G50A L56T	E54K A57N S58E H61F	L99T A100V	$2.94x10^{-3}$	163.3	$5.39x10^{-4}$	3.37
M47	R28N	G50A L56T	E54K A57N S58G	L99T A100V	$8.23x10^{-3}$	457.2	$3.32x10^{-4}$	2.08
M48	R28N	G50A L56T	E54K A57N S58L	L99T A100V	0.0343	1905.6	$8.42x10^{-4}$	5.26
M49	R28N	G50A L56T	E54K A57N S58Y H61F	L99T A100V	0.0148	822.2	$5.95x10^{-4}$	3.72
M50	R28N	G50A L56T	E54K A57R	L99T A100V	$5.30x10^{-3}$	294.4	$4.06x10^{-4}$	2.54
M51	R28N	L56I	E54K A57G	L99T A100V	$1.18x10^{-3}$	65.6	$1.31x10^{-4}$	0.82
M52	R28N	L56I	E54K A57N S58A	L99T A100V	$2.29x10^{-3}$	127.2	$2.81x10^{-4}$	1.76
M53	R28N	L56I	E54K A57N S58G	L99T A100V	$1.91x10^{-3}$	106.1	$3.74x10^{-4}$	2.34
M54	R28N T30A	G50A	E54K A57N S58P	L99T A100V	$2.16x10^{-3}$	120.0	$1.79x10^{-3}$	11.19
M55	R28N T30A	L56S	E54K A57N S58E E64D	L99T A100V	$5.85x10^{-3}$	325.0	$4.78x10^{-4}$	2.99
M56	R28N T30D	L56S	E54K A57N H61F	L99T A100V	$9.35x10^{-3}$	519.4	$4.79x10^{-4}$	2.99
M57	R28N T30D	L56S	E54K A57N	L99T A100V	0.0104	1,200	$3.22x10^{-4}$	3.08

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
			S58E					
M58	R28N T30D	L56S	E54K A57N S58I H61F	L99T A100V	No binding	n.d.	$1.95x10^{-3}$	12.19
M59	R28N T30D	L56S	E54K A57N S58N H61F	L99T A100V	0.0123	683.3	$5.24x10^{-4}$	3.28
M60	R28N T30D	L56S	E54K A57N S58R H61F	L99T A100V	0.0272	1511.1	$9.11x10^{-4}$	5.69
M61	R28N T30G	A51H	E54Q A57N H61F	L99T A100V	$5.21x10^{-3}$	289.4	$4.59x10^{-4}$	2.87
M62	R28N T30G	A51H L56T	E54K A57N S58E	L99T A100V	$5.75x10^{-3}$	242	$5.57x10^{-4}$	5.86
M63	R28N T30G	G50A	E54K A57N S58T	L99T A100V	$2.65x10^{-3}$	147.2	$1.50x10^{-3}$	9.38
M64	R28N T30G	G50A	E54K A57N S58V	L99T A100V	0.0234	1300.0	$1.32x10^{-3}$	8.25
M65	R28N T30G	G50A L56I	E54K A57C	L99T A100V	$4.07x10^{-3}$	226.1	$8.03x10^{-4}$	5.02
M66	R28N T30G	L56I	E54K A57E	L99T A100V	$5.11x10^{-3}$	283.9	$5.20x10^{-4}$	3.25
M67	R28N T30G	L56I	E54K A57F	L99T A100V	$1.71x10^{-3}$	95.0	$8.20x10^{-4}$	5.13
M68	R28N T30G	L56I	E54K A57N S58D E64D	L99T A100V	$6.76x10^{-3}$	375.6	$4.28x10^{-4}$	2.68
M69	R28N T30G	L56I	E54K A57N S58E	L99T A100V	$1.81x10^{-3}$	100.6	$7.33x10^{-4}$	4.58
M70	R28N T30G	L56I	E54K A57S	L99T A100V	$6.07x10^{-3}$	337.2	$5.59x10^{-4}$	3.49
M71	R28N T30G	L56I	E54K A57Y	L99T A100V	$2.12x10^{-3}$	117.8	$1.28x10^{-3}$	8.00
M72	R28N T30G	L56S	E54K	L99T A100V	$3.95x10^{-3}$	219.4	$4.00x10^{-4}$	2.50
M73	R28N T30G	L56S	E54K A57N S58Y E64D	L99T A100V	$3.00x10^{-3}$	166.7	$2.55x10^{-4}$	1.59
M74	R28N T30G	L56S	E54K A57S	L99T A100V	$6.03x10^{-3}$	335.0	$5.97x10^{-4}$	3.73
M75	R28N	L56S	E54K	L99T	$1.87x10^{-2}$	1038.9	$1.16x10^{-3}$	7.25

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
	T30G		A57V	A100V				
M76	R28N T30S	G50A L56T	A57G	L99T A100V	$1.16x10^{-3}$	64.4	$3.64x10^{-4}$	2.28
M77	R28N T30S	G50A L56T	E54K A57D	L99T A100V	0.0143	794.4	$4.77x10^{-4}$	2.98
M78	R28N T30S	G50A L56T	E54K A57N S58T	L99T A100V	0.167	9277.8	$1.31x10^{-3}$	8.19
M79	R28N T30S	G50A L56T	E54K A57P	L99T A100V	0.19	10555.6	$1.29x10^{-3}$	8.06
M80	R28N T30S	L56I	E54K A57N S58V	L99T A100V	0.0993	5516.7	$2.09x10^{-3}$	13.06
M81	R28N T30S	L56S	E54K A57N S58E	L99T A100V	$4.29x10^{-3}$	238.3	$4.90x10^{-4}$	3.06
M82	R28N T30V	A51H L56T	A57N	L99T A100V	$6.99x10^{-3}$	388.3	$8.77x10^{-4}$	5.48
M83	R28N T30V	A51H L56T	E54K A57N S58M H61F	L99T A100V	No binding	n.d.	$9.33x10^{-4}$	5.83
M84	R28N T30V	A51H L56T	E54N A57N	L99T A100V	$1.76x10^{-2}$	977.8	$1.08x10^{-3}$	6.75

All CDRs including both Kabat and Chothia CDRs. Amino acid residues are numbered sequentially (see Figure 5). All clones have L3+H1+H3 sequences identical to G1.

$K_D = k_{off}/k_{on}$. All k_{off} values were determined in a screening mode except those that are underlined, which were obtained by global analysis of a Fab concentration series (G1 was analyzed in a high-resolution mode). Underlined K_D values were therefore determined experimentally by measuring k_{on}. Other k_{on} values were estimated to be the same as M25.

n.d. = not determined

To determine the epitope on human α-CGRP that is recognized by antibody G1, Biacore assays described above were used. Human α-CGRP was purchased as an N-biotinylated version to enable its high-affinity capture via SA sensor chips. The binding of G1 Fab fragment to the human α-CGRP on the chip in the absence or presence of a CGRP peptide was determined. Typically, a 2000:1 mol peptide/Fab solution (e.g., 10 μM peptide in 50nM G1 Fab) was injected across human α-CGRP on the chip. Figure 6 shows the percentage of binding blocked by competing peptide.

Data shown in Figure 6 indicate that peptides that block 100% binding of G1 Fab to human α-CGRP are 1-37 (WT), 8-37, 26-37, P29A (19-37), K35A (19-37), K35E (19-37), and K35M (19-37) of human α-CGRP; 1-37 of β-CGRP (WT); 1-37 of rat α-CGRP (WT); and 1-37 of rat β-CGRP (WT). All these peptides are amidated at the C-terminus. Peptides F37A (19-37) and 19-37 (the latter not amidated at the C-terminus) of human α-CGRP also blocked about 80% to 90% of binding of G1 Fab to human α-CGRP. Peptide 1-36 (not amidated at the C-terminus) of human α-CGRP blocked about 40% of binding of G1 Fab to human α-CGRP. Peptide fragment 19-36 (amidated at the C-terminus) of human α-CGRP; peptide fragments 1-13 and 1-19 of human α-CGRP (neither of which are amidated at the C-terminus); and human amylin, calcitonin, and adrenomedullin (all amidated at the C-terminus) did not compete with binding of G1 Fab to human α-CGRP on the chip. These data demonstrate that G1 targets a C-terminal epitope of CGRP and that both the identity of the most terminal residue (F37) and its amidation is important for binding.

Binding affinities of G1 Fab to variants of human α-CGRP (at 37°C) was also determined. Table 7 below shows the affinities as measured directly by titrating G1 Fab across N-biotinylated human α-CGRP and variants on the chip. Data in Table 7 indicate that antibody G1 binds to a C-terminal epitope with F37 and G33 being the most important residues. G1 does not bind to CGRP when an extra amino acid residue (alanine) is added at the C-terminal (which is amidated).

Table 7. Binding affinities of G1 Fab to human α-CGRP and variants measured at 37°C (see Table 4 for their amino acid sequences)

CGRP on chip	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
1-37 (WT)	$4.68x10^5$	$7.63x10^{-5}$	0.16 (high resolution K_D = 0.06)
19-37	$4.60x10^5$	$7.30x10^{-5}$	0.16
25-37	$3.10x10^5$	$8.80x10^{-5}$	0.28
F27A (25-37)	$3.25x10^5$	$1.24x10^{-4}$	0.38
V28A (25-37)	$3.32x10^5$	$9.38x10^{-5}$	0.28
P29A (25-37)	$2.26x10^5$	$1.78x10^{-4}$	0.79
T30A (25-37)	$1.79x10^5$	$8.41x10^{-5}$	0.47
N31A (25-37)	$2.17x10^5$	$1.14x10^{-4}$	0.53
V32A (25-37)	$2.02x10^5$	$3.46x10^{-4}$	1.71
G33A (25-37)	$2.07x10^5$	0.0291	141
S34A (25-37)	$2.51x10^5$	$7.64x10^{-4}$	3.04

CGRP on chip	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
K35A (19-37)	$2.23x10^5$	$2.97x10^{-4}$	1.33
K35E (19-37)	$5.95x10^4$	$5.79x10^{-4}$	9.73
K35M (19-37)	$2.63x10^5$	$1.34x10^{-4}$	0.51
K35Q (19-37)	$1.95x10^5$	$2.70x10^{-4}$	1.38
F37A (25-37)	$8.90x10^4$	$8.48x10^{-3}$	95 (solution K_D = 172 nM)
38A (25-38A)	-	-	No binding detected

The above data indicate that the epitope that antibody G1 binds is on the C-terminal end of human α-CGRP, and amino acids 33 and 37 on human α-CGRP are important for binding of antibody G1. Also, the amidation of residue F37 is important for binding.

Example 5: Effect of anti-CGRP antagonist antibody G1 on skin vasodilatation induced by stimulation of rat saphenous nerve

To test antagonist activity of anti-CGRP antibody G1, effect of the antibody on skin vasodilatation by stimulation of rat saphenous nerve was tested using a rat model described in Example 3. Briefly, rats were maintained anesthesia with 2% isoflurane. Bretylium tosylate (30 mg/kg, administered i.v.) was given at the beginning of the experiment to minimize vasoconstriction due to the concomitant stimulation of sympathetic fibers of the saphenous nerve. Body temperature was maintained at 37°C by the use of a rectal probe thermostatically connected to a temperature controlled heating blanket. The saphenous nerve of the right hindlimb was exposed surgically, cut proximally and covered with plastic wrap to prevent drying. A laser Doppler probe was placed over the medio-dorsal side of the hindpaw skin, which is the region innervated by the saphenous nerve. Skin blood flow, measured as blood cell flux, was monitored with a laser Doppler flow meter. In experiments to determine effects of antibody within two hours of injection thirty to forty-five minutes after bretylium tosylate injection, when a stable base-line flux (less than 5% variation) was established for at least 5 minutes, the nerve was placed over platinum bipolar electrodes and electrically stimulated (2Hz, 10V, 1 ms, for 30 seconds) and again 20 minutes later. The average of the blood flow flux response to these two stimulations was used to establish the baseline response (time 0) to electrical stimulation. Antibody G1 (1 mg/kg or 10 mg/kg) or vehicle (PBS with 0.01%

Tween 20 equal volume to 10 mg/kg G1) were then administered intravenously (i.v.). The nerve was subsequently stimulated (2Hz, 10V, 1 ms, for 30 seconds) at 30 minutes, 60 minutes, 90 minutes, and 120 minutes after the antibody administration. Animals were kept under anesthesia for a period of approximately three hours. Cumulative change in skin blood flow was estimated by the area under the flux-time curve (AUC, which is equal to change in flux multiplied by change in time) for each flux response to electrical pulse stimulations.

As shown in Figure 7, blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody G1 at 1 mg/kg (administered i.v.) as compared to the vehicle, when the saphenous nerve was electrically stimulated at 90 min after the antibody administration. Blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody G1 at 10 mg/kg (administered i.v.) as compared to the vehicle, when the saphenous nerve was electrically stimulated at 90 minutes and 120 minutes after antibody administration.

In experiments to determine effects of the antibodies at longer time points in the saphenous assay, rats were injected i.v. with the indicated doses of antibody 24 hours or 7 days prior to preparing the animal for saphenous nerve stimulation as described above. In these experiments it was impossible to establish a baseline response in individual rats to electrical pulse stimulation prior to dosing, so treated groups were compared to animals dosed with vehicle (PBS, 0.01% Tween 20) at 24 hours or 7 days.

As shown in Figures 8A and 8B, blood flow increases in the dorso-medial hindpaw skin evoked by saphenous nerve stimulation were significantly inhibited in the groups of animals dosed with either 10 mg/kg or 3 mg/kg G1 at either 24 hours or 7 days prior to stimulation as compared to vehicle groups dosed at the same time points.

Figure 8C represents a curve fit analysis applied to the dose response data represented in figures 8A and 8B to determine the dose required for 50% maximal effect (EC_{50}). The EC_{50} at 24 hours is 1.3 mg/kg and the EC_{50} at 7 days is slightly lower (0.8 mg/kg).

Example 6: Acute effect of anti-CGRP antagonist antibody mu7E9 in a dural artery (closed cranial window) assay

Closed Cranial Window Model: The purpose of this experiment was to determine the acute effect of anti-CGRP antagonist antibodies and compare it with the acute effect of the CGRP receptor antagonist BIBN4096BS. Experiments were carried out as previously described (Williamson et al., Cephalalgia 17(4):518-24 (1997)) with the following modifications. Sprague Dawley rats (300-400g) were anesthetized with 70 mg/kg i.p. pentobarbital. Anesthesia was maintained with 20 mg/kg/hr i.v. pentobarbital. Rats were cannulated through the jugular vein for delivery of all drugs. Blood pressure was monitored with a probe (mikro-tip catheter, Millar Instruments) threaded through the femoral artery into the abdominal aorta. The rats were tracheotomized and breathing rate was maintained at 75 breaths per minute at a volume of 3.5 mL. After fixating the head in a stereotactic instrument and removing the scalp, a 2x6 mm window in the left parietal area just lateral to the sagittal suture was made by thinning the bone with a dental drill. Using a micromanipulator, a platinum bipolar electrode was lowered onto the surface and covered with heavy mineral oil. Lateral to the electrode window another window of 5x6 mm was created and filled with heavy mineral oil through which the diameter of a branch of the middle meningeal artery (MMA) was continuously monitored with a CCD camera and a video dimension analyzer (Living Systems). The rats were rested for no less than 45 minutes after the preparation. A baseline response to electrical stimulation was established (15 V, 10 hz, 0.5 ms pulses, 30 seconds) and then rats were dosed i.v. with experimental compound (10 mg/kg mu7E9, 300 µg/kg BIBN4096BS, or PBS 0.01%, Tween 20). Additional electrical stimulations were done at 5 (BIBN4096BS), 30, 60, 90, and 120 minutes after dosing. All data was recorded using chart software (ADInstruments).

As shown in Figure 9, mu7E9 at 10 mg/kg significantly blocks MMA dilation evoked by electrical field stimulation within 60 minutes after dosing and maintains the effect throughout the duration of the assay (120 minutes). For comparison BIBN4096BS blocks MMA dilation within 5 minutes of dosing but the effect has

completely disappeared by 90 minutes. The magnitude of the block is comparable between BIBN4096BS and mu7E9.

Example 7: Chronic effect of anti-CGRP antagonist antibody G1 in a dural artery (closed cranial window) assay

The purpose of this experiment was to determine if the anti CGRP antibody could still block electrically stimulated MMA dilation 7 days after dosing. Preparation of the rats was identical to the above described acute experiment (Example 6) with the following exceptions. Rats were injected i.v. (10 mg/kg, 3 mg/kg, or 1 mg/kg G1) 7 days prior to creating the closed cranial window prep and stimulation. It was impossible to establish a baseline dilation response to electrical stimulation prior to dosing as in the acute experiment so the antibody groups were compared to dilation of the MMA in a vehicle (PBS, 0.01% Tween 20) dosed control group. After the rats were allowed to rest for no less than 45 minutes the dura was electrically stimulated at 30 minute intervals. Stimulations were at 2.5V, 5V, 10V, 15V, and 20V, all at 10 Hz, 0.5 ms pulses for 30 seconds.

As shown in Figure 10, G1 at 10 mg/kg and 3 mg/kg significantly blocked MMA dilation evoked by electrical stimulation in the range of 10 to 20 volts. This data demonstrates that G1 can block electrically stimulated MMA dilation up to 7 days after dosing.

Example 8: Morphine withdrawal hot flush model

The morphine withdrawal rat model is an established rodent model for menopausal hot flush mechanisms (Sipe et al., Brain Res. 1028(2):191-202 (2004); Merchenthaler et al., Maturitas 30:307-316 (1998); Katovich et al., Brain Res. 494:85-94 (1989); Simpkins et al., Life Sciences 32:1957-1966 (1983)). Basically the rats are addicted to morphine by implanting morphine pellets under the skin. Upon addiction the animals are injected with naloxone (opioid antagonist) which sends them into withdrawal immediately. This withdrawal is accompanied by a skin temperature increase, a core body temperature decrease, an increase in heart rate and an increase in serum

luteinizing hormone. These are all similar in magnitude and timing to what occurs in human hot flush (Simpkins et al., Life Sciences 32:1957-1966 (1983)). Furthermore, if rats are treated with estradiol prior to inducing withdrawal, the symptoms of hot flush are reduced (Merchenthaler et al., Maturitas 30:307-316 (1998)). This is why the morphine withdrawal model is believed to mimic clinical hot flush.

Ovariectomized rats were ordered from Charles River Laboratories. Not less than 7 days post ovariectomy morphine dependency was created by implanting a morphine pellet (75 mg morphine base) subcutaneously. Two days later, two more pellets were implanted. The following day rats were injected intravenously with either 10 mg/kg 4901 [**] or vehicle (PBS, 0.01% tween). Two days after the second pelleting the rats were anesthetized with ketamine (90 mg/kg) and lightly restrained. A surface temperature thermocouple was taped to the base of the tail and a rectal thermocouple is used to measure core temperature. Data was recorded using Chart software (ADInstruments). After recording 15 minutes of stable baseline temperature, naloxone (1mg/kg) was injected subcutaneously. Temperature was recorded continuously for the next 60 minutes. The results are shown in Figures 11A and 11B.

Example 9: Non-clinical Toxicology and Pharmacokinetics

Anti-CGRP antagonist antibody G1 was well-tolerated in 1-month IV repeat-dose toxicity studies in Sprague-Dawley (SD) rats and cynomolgus monkeys and no target organ toxicity was determined in either of these studies. A no adverse event level (NOAEL) of 100 mg/kg/week was established for both the rat and monkey studies. This dose level corresponded to systemic exposure with a maximum concentration (Cmax) of 2,570 and 3,440 µg/mL and areas under the curve (AUC(0-168h)) of 194,000 µg•h/mL and 299,000 µg•h/mL (Day 22) in rats and monkeys, respectively.

In a 3-month IV/SC rat study, no target organ toxicities were identified and G1 was well-tolerated up to the highest tested dose, 300 mg/kg. In a 3-month monkey study, perivascular inflammation of the ciliary artery, as the result of the deposition of immune complexes was observed at ≥100 mg/kg. This finding was attributed to the monkey's immunogenic response to a humanized antibody and was not considered to

be clinically relevant. The highest tested dose of 300 mg/kg in this monkey study are at least 10-fold greater than the highest anticipated clinical dose of 2,000 mg or 29 mg/kg on a mg/kg basis (assuming an average subject weight of 70 kg).

Example 10: Clinical Pharmacokinetics

The PK of antibody G1 following single IV exposure was examined in four randomized, placebo-controlled, double-blind studies examining doses between 10 and 2,000 mg. Maximum plasma concentrations (Cmax) were reached shortly after the end of the 1-hour IV infusion. Median time to Cmax (Tmax) ranged from 1.0 to 3.0 hours, followed by a multiphasic decline. Cmax and total exposure increased approximately linearly with escalating doses of G1. Terminal half-life (t½) ranged from 36.4 to 48.3 days. There is no evidence of G1 metabolism in the liver, the primary mode of metabolism is by proteosomic degradation.

One study defined the pharmacokinetics of 30 mg and 300 mg doses given twice, two weeks apart. Maximum concentrations and area under the concentration-time profile increased with increasing dose. The apparent terminal half-life (t½) after the second dose was 41.2 days (30 mg) and 50.0 days (300 mg) (arithmetic mean). The plasma accumulation ratios of G1 after two IV doses administered 15 days apart were 1.5 (30 mg) and 1.4 (300 mg).

Example 11: Clinical Safety and Pharmacokinetics

In six studies, antibody G1 was administered to 118 healthy males and females, while 57 male and female subjects received placebo. The study included single IV doses ranging from 0.2 mg up to 2,000 mg, two IV doses of up to 300 mg given once every 14 days, and SC administration of 225 and 900 mg. The six studies included: two IV single dose escalation PK and pharmacodynamics (PD) studies in healthy males (studies B0141001 and B0141002); a two-cohort, placebo controlled cross-over study to examine the acute effects of IV administration of antibody G1 on capsaicin flare response in healthy volunteers (B0141006); a parallel group repeat dose study of antibody G1 in healthy male and female volunteers (B0141007); a single dose study

evaluating the safety and tolerability of doses up to 2,000 mg administered IV to healthy female volunteers (B0141008), and a study comparing the relative safety and bioavailability between IV and SC administration (G1-SC-IV).

The six studies are summarized below in Table 8. Of the five IV studies (B0141001, B0141002, B0141006, B0141007 and B0141008), three had virtually identical designs and assessments. Study B014100 tested doses of 0.2 mg, 1 mg, and 3 mg given as a single one-hour IV infusion. The study had a parallel design. Participants were confined in the clinic for seven days after the infusion, with multiple assessments on each of these days. After discharge, patients were reassessed one week after discharge (day 14), and then one, two, and three months after the infusion. Study B0141002 tested doses ranging from 10 mg to 1000 mg as a single administration. Finally, Study B0141008 tested doses of 300 mg, 1000 mg, 1500 mg, or 2000 mg. Study B0141006 was distinct from the others since it also aimed to integrate pharmacodynamic readouts through measuring capsaicin flare inhibition up to one week after IV infusion of antibody G1.

For the IV studies, adverse events (AEs) profiles were reported for the first dosed period only. Study B0141007 tested multiple doses of antibody G1 at either 30 or 300 mg IV given two weeks apart, using a parallel design. Each eligible subject was assigned a randomization sequence via an interactive Web-based system that contained the treatment assignment. The randomization schema was developed by the lead statistician. Participants in all studies were generally healthy men and women (from 18 to 65 years of age); all participants signed informed consent forms. All studies were approved by investigation review boards (IRBs). AEs were defined as any untoward medical occurrence in clinical study participants, with or without causal relationship to study drug. AEs observed after administration of the study drug or placebo were termed "treatment-emergent" AE (TEAEs) regardless of potential causality with the study drug. All subjects experiencing TEAEs were followed at appropriate time intervals until the event had resolved or until the event had stabilized and/or reached a new baseline. All TEAEs were ranked as being mild, moderate, or severe. Serious AEs (SAEs) were defined a priori as any untoward medical occurrence

that at any dose resulted in death, was life threatening (i.e., the subject was at immediate risk of death at the time of the event), required inpatient hospitalization or prolongation of existing hospitalization, resulted in persistent or significant disability/incapacity (e.g., a substantial disruption of the subject's ability to carry out normal life functions), resulted in a congenital anomaly/birth defect, or any other medically important event. Treatment-related AE (TRAEs) were to be considered when one of the following situations was present: 1) a plausible temporal relationship between the onset of the AE and administration of the investigational product could be identified; 2) the AE could not be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and 3) the AE abated on discontinuation of the investigational product or dose reduction.

Blood pressure, pulse rate and oral temperature were measured at screening, pre-dose, immediately after the end of the infusion and multiple times during the patients' confinements in the clinics, as well as at all clinic visits. Laboratory tests included serum chemistries, hematology, and urinalysis. Hematology, chemistry, coagulation, and urine safety laboratory tests were performed at multiple study times. ECGs were recorded at screening, pre-dose on Day 1, immediately after the end of the infusion and five other times during the first day, as well as in all clinic visits. QTcF values were derived using Fridericia's (QTcF) heart rate correction formula. Absolute values and changes from baseline for the ECG parameters QT interval, heart rate, QTcF interval, PR interval and QRS interval were assessed by cohort, treatment, and time post-dose. In addition to the safety assessments described above, Protocol B014008 included complete ophthalmic assessments at baseline and at three time points after dosing (Day 28, Day 84, and Day 168).

Clinical data and vital signs were summarized using descriptive tables and summary statistics. Laboratory and other safety data were summarized as a function of any change (values outside of the reference range), as well as any clinical relevant changes, which were defined a priori. Summary tables were stratified by dose and data were pooled across studies. In addition, comparisons for consolidated data for all antibody G1 exposures were contrasted with placebo. Placebo was also contrasted

with antibody G1 doses of 100 mg and higher (100 mg, 300 mg, 1000 mg, 1500 mg, and 2000 mg), and with antibody G1 doses of 1000 mg and higher (1000 mg, 1500 mg, and 2000 mg).

In the IV/SC study (G1-SC-IV), thirty-six subjects were randomized to receive a single administration of antibody G1 (225 or 900 mg) or placebo, delivered as either a subcutaneous (SC) bolus injection or a 1-hour IV infusion. Subjects were confined in the clinical research unit for seven days after dosing, and returned to the clinic periodically for additional outpatient visits up to Study Day 90. ECGs were performed extensively on Day 1 (pre-dose, Hours 1, 6, 12), Day 3, and Day 7 while the subjects were confined and once at the completion of the study (Day 90). Vital signs, including temperature, blood pressure and heart rate, were collected pre-dose, Days 1, 3, 7, and 90.

Table 8

Study	Study population	Treatment with Antibody G1
B0141001	Healthy adult men (n = 24)	Single intravenous (IV) infusion of 0.2, 1 or 3 mg in cohorts of eight (six/cohort, active treatment; two in placebo cohort)
B0141002	Healthy adult men (n = 40)	Single IV infusion of 10, 30, 100, 300 or 1000 mg in cohorts of eight (six/cohort, active treatment; 10 in placebo cohort)
B0141006	Healthy adult men (n = 12)	Two cohorts modified cross-over, placebo or 300 mg IV infusion in cohorts of six. In the first period, all participants received placebo. For the second period (included herein), 12 participants received placebo and 11 received 300 mg.
B0141007	Healthy adult men and women (n = 21)	Two IV infusions two weeks apart at 30 or 300 mg in cohorts of 10 or 11 (six/cohort, active treatment; nine in placebo cohort)
B0141008	Healthy adult women (n = 31)	Single IV infusion of 300, 1000, 1500, or 2000 mg (five in 2000 mg cohort; six/cohort remaining treatment groups; eight in placebo cohort)
G1-SC-IV	Thirty-six subjects (n = 36)	Single subcutaneous (SC) bolus injection or single IV infusion of 225 or 900 mg

Across the broad range of dosages evaluated in the five IV studies (0.2 to 2,000 mg), IV antibody G1 was acceptably tolerated. Table 9 summarizes the overall adverse event (AE) rate by dose for the IV studies. Based on these tolerability results,

overt safety concerns have not emerged. Across all trials in the IV studies, participants receiving placebo reported an average of 1.3 treatment emergent adverse events (TEAEs). These are all reported events, regardless of the investigator's opinion of relationship to study drug. Across all IV G1 doses, the rate was 1.4 TEAEs/subject. Subjects receiving G1 doses of 100 mg or higher had an average of 1.5 TEAEs; those receiving doses of 1,000 mg or higher had an average of 1.6 TEAEs.

Table 9

	Subjects Evaluated for AE	Number of AEs – n (N)	Subjects with AE – n (N)	Subject with Serious AE – n (N)	Subject with Severe AE – n(N)	Subjects discontinued for AEs – n (N)	Dose reduced or temporary discontinuations – n (N)
Placebo	45	57 (11)	23 (8)	0	0	2 (1)	0
0.2mg	6	5 (0)	2 (0)	0	0	0	0
1mg	6	1 (1)	3 (0)	0	0	0	0
3mg	6	10 (2)	4 (1)	0	0	0	0
10mg	6	5 (1)	4 (1)	0	0	0	0
30mg	12	21 (11)	8 (5)	0	0	0	0
100mg	6	5 (1)	4 (1)	0	0	0	0
300mg	29	47 (10)	20 (7)	1 (1)	1 (1)	0	0
1000mg	12	17 (4)	8 (4)	0	0	0	0
1500mg	6	8 (0)	3 (0)	0	1 (0)	0	0
2000mg	5	12 (2)	4 (1)	0	0	0	1 (1)

AE = adverse events; n= any event, treatment related or not; (N) = considered treatment related by investigator. Note: For protocol B0141006 (placebo and 300 mg), only data for the first active treatment period was included, due to its cross-over nature

In the IV studies, treatment-related adverse events (TRAEs, or AEs that might be related to the therapy according to the primary investigator) were reported in 21.2% of subjects receiving IV G1, compared to 17.7% in those receiving placebo. At doses of 100 mg of G1 or higher, TRAEs occurred in 22.4% of participants. At doses of 1,000 mg or higher, TRAEs occurred in 21.7% of participants. Antibody G1 does not appear to be associated with any clinically relevant patterns of change in vital signs (systolic and diastolic blood pressure [BP], temperature and heart rate [HR]), electrocardiogram (ECG) abnormalities (including QTcB and QTcF), infusion site reactions, or clinical laboratory findings. There were limited effects on liver function tests (aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin, and alkaline

phosphatase) with a grade 1 increase in total bilirubin in one subject receiving placebo (Study B0141001), and a grade 1 increase in ALT in one subject receiving placebo (Study B0141002). Clinically significant liver function abnormalities were not seen among subjects receiving any of the studied doses of G1. There was no evidence of differences between G1 and placebo in hematological tests assessing renal function, electrolytes, or in urine tests.

In the IV/SC study (G1-SC-IV), safety and tolerability were comparable between SC and IV routes of delivery. Mean heart rate and blood pressure (diastolic and systolic) were not affected by antibody G1 treatment, nor were there any meaningful changes in any cardiovascular parameter after treatment with SC antibody G1. A summary of TRAEs observed during the SC study is shown below in Table 10.

Table 10

	900 mg (N=6)	**225 mg (N=6)**	**Placebo (N=6)**
GI disorders	2 (33.3%)	0	1 (16.7%)
CNS	0	1 (16.7%)	0
Infections and Infestations	0	0	0
Musculoskeletal and connective tissue	0	0	0
Respiratory	0	0	0
Reproductive and Breast Disorders	0	0	0
Injuries	0	0	0
Pregnancy	0	0	0
Renal	1 (16.7%)	0	0
Vascular	0	0	0

In the single dose studies (B0141001, B0141002, B0141006, and B0141008), pharmacokinetic (PK) parameters were calculated for doses ranging from 30 mg to 2,000 mg. Group mean terminal half-life ($t_{1/2}$) ranged from approximately 40 to 48 days. C_{max} and total exposure (assessed by AUC_{inf}) increased with increasing dose. The increase in AUC_{inf} appeared to be approximately dose proportional between 30 and 1,000 mg and appeared to be greater than dose proportional between 1,000 and 2,000 mg. The volume of distribution was low, between 6-10 L.

In the two dose study (B0141007), the apparent terminal half-life after a second dose was between 41 and 50 days. Plasma concentrations accumulated after the second dose, with an accumulation ratio of approximately 1.5. Moreover, in the IV/SC study (G1-SC-IV), pharmacokinetic assessments indicated G1 had a similar terminal half-life when delivered SC as IV.

Example 12: Non-clinical safety

Two studies assessing the safety of antibody G1 were conducted in cynomolgus monkeys. In the first study, safety of a single dose of antibody G1 was evaluated. In the second study, safety of repeated dosing of antibody G1 was evaluated. Each of the studies and their results are further described in detail below. For both the single and repeat-dose studies, antibody G1 was formulated as a 51.4 mg/mL solution in 20 mM histidine, 84 mg/mL trehalose dihydrate, 0.2 mg/mL polysorbate 80, 0.05 mg/mL disodium EDTA dihydrate and 0.1 mg/mL L-methionine, pH ~5.5. Vehicle was formulated identically without antibody G1. Additionally, in both studies, blood samples were taken periodically for analysis of antibody G1 plasma concentration using a validated ELISA method.

Data were first aggregated in summary tables and figures using GraphPad Prism (version 6.0) and Excel 2010 (Microsoft). For the single exposure study, telemetry data were analyzed using ANOVA. Analysis was performed using SAS Release 8.2. In order to normalize the QT interval over a range of R-R intervals, Individual Animal Correction Factors (IACFs) were generated for each animal by relating each RR-interval with its associated QT-interval. The linear regression of this QT/RR-interval relationship was determined for the data set. The slope of this linear regression was used as the IACF for the associated animal across all treatments. This IACF was used to calculate the corrected QT-interval (QTc) using the following equation:

QT-I(c) = QT interval corrected for heart rate = QT-I - [(RR - 300)*(IACF)].

For the multiple-dose study, one-way ANOVA was also used to analyze data. If the ANOVA was significant ($P \leq 0.05$), Dunnett's post-test was used for in-between group comparisons. For each gender, the treated group was compared with the control (vehicle) group at the 5% two-tailed probability level.

Single-dose telemetry study

Eight adult male cynomolgus monkeys (Charles River Primates) were surgically instrumented with telemeters and allowed to recover for at least two weeks. Implants (DSI TL11M2-D70-PCT) and receivers (RMC-1) were manufactured by Data Sciences International.

Animals were acclimated to telemetry data acquisition cages at least overnight prior to dosing. During acclimation, pre-study recording of hemodynamic parameters was conducted to verify that the transducers and equipment were functioning correctly. During telemetered data acquisition, animals were housed individually in cages equipped with telemetry receivers. On non-collection days, animals were housed in cages without telemetry receivers. Animals were maintained on a 12-hours light, 12-hours dark day cycle, with ad libitum water and fed with certified primate diet.

For the first phase of the study, animals (8 males) were administered vehicle only, and telemetry data were collected beginning ~1 hour pre-dose through 22 hours post-dose. Six days after vehicle administration, the same animals received a single IV administration of antibody G1 (100 mg/kg, an ~10-fold greater dose than the pharmacological EC50 in cynomolgus monkeys). Telemetered electrocardiographic and hemodynamic data were again continuously recorded from all animals. In addition, these animals were monitored for ~24 hours on days 3, 7, 10, and 14 after receiving their single dose of antibody G1. Telemetered ECG and blood pressure signals were transmitted via the implanted radio-telemetry devices to receivers mounted in each cage. The acquired signals were passed through a data exchange matrix (DSI model DEM) and on to a PC-based data acquisition system (DSI software Ponemah P3 version 3.4); the data analysis software was Emka Technologies version 2.4.0.20 (Emka Technologies). The analog/digital sampling rate was 1,000 Hz for telemetered

ECG data and 500 Hz for blood pressure data. Data were logged as one minute means.

Group mean systolic blood pressure (SBP) was similar before and after treatment with antibody G1 throughout the first day after dosing and on subsequent days (animals telemetered on days 3, 7, 10 and 14 at identical time intervals as day 1). At hours 1–4 post-dosing when antibody G1 blood concentrations were at maximal levels (mean concentration of 3,500 µg/mL at 4 hours), mean SBP was 111 mmHg compared with 113 mmHg at the same time interval following vehicle administration. Furthermore, SBP was 110 mmHg on days 3 and 7, 109 mmHg on day 10 and 110 mmHg on day 14 after antibody G1 administration. Similar SBP data were recorded for other time intervals. Since this was a crossover designed study, the treated animals served as their own controls. When the data were analyzed as differences in blood pressure after antibody G1 administration compared with vehicle treatment, there are minor statistically significant reductions in SBP at the latter time interval on days 7, 10, and 14.

Following treatment with antibody G1, diastolic blood pressure (DBP) was noted to be around 3 mmHg lower than the mean values obtained after vehicle administration. From hours 5–22, the group mean for the vehicle and antibody G1 group were similar. The same trend was seen on other days, when a slight decrease in the DBP (ranging from 2.62–3.5 mmHg) occurred in the first interval measured, with a few changes of similar magnitude seen sporadically on days 7–10 in the 7–22 hour interval. Similar to what was seen for the DBP, minor decreases in the heart rate were seen during the first assessment (hours 1–4) relative to vehicle treatment. Differences were undetectable during the intermediate assessments and were once more seen between hours 18–22 on all days.

Moreover, with respect to ECG findings, there were no statistically significant changes in QTc interval at any time point, relative to vehicle treatment. Although statistically significant changes in RR, PR, RS and QT were seen over the 14 day period when compared with vehicle, they were all minor in absolute value.

Repeat-dose safety study

The repeat-dose safety study included 48 adult, gender-matched (6 per gender per group) antibody G1-naïve cynomolgus monkeys (Charles River Primates). Animals received vehicle or antibody G1 as an intravenous injection once weekly for 14 weeks at doses of 10 mg/kg, 100 mg/kg, or 300 mg/kg. In each group, two animals of each gender were allowed to recover for an additional 4 months following the end of dosing.

ECG and blood pressure measurements were recorded once during the pre-study phase, twice after steady-state was achieved (prior to dosing and 4 hours post-dose on Day 85) and once ~1 week after the end of dosing (day 103 of the recovery phase). Animals were anesthetized with ketamine and ECGs were recorded using eight leads. Measurement of ECGs (including heart rate) was done with the captured data using the Life Science Suite Ponemah Physiology Platform software system via DSI, using leads I, II, aVF, CG4RL and CV4LL, as standard. A heart rate correction for the QT interval (QTc) was calculated using the Bazett formula.

Blood pressure was recorded prior to the first dose, after 12 weeks of dosing (13 doses) and approximately 1 week after the end of dosing. No significant changes were noted in SBP or DBP in any of the treated groups of animals relative to vehicle-treated animals. Group mean heart rates were relatively consistent across the dose groups and time points measured, with no statistical differences measured. Plasma concentrations of antibody G1 were measured during the first week of dosing and at the time of blood pressure and ECG assessments, demonstrating accumulation with repeated, weekly dosing.

Moreover, with respect to ECG findings, there were no significant differences in QTc interval across all doses and time points. Additionally, no significant or relevant ECG changes were seen for any of the ECG parameters assessed over the course of the study.

In summary, antibody G1 was very well tolerated in both studies, with no clinically significant changes noted in any hemodynamic parameter, nor any relevant changes noted in any ECG parameter. In cynomolgus monkeys, cardiovascular and

hemodynamic parameters do not appear to be affected by long-term inhibition of CGRP with antibody G1.

Example 13: Phase 2 study

A Phase 2, Multicenter, Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study Comparing the Efficacy and Safety of 4 Dose Regimens of Subcutaneous Administration of antibody G1 (TEV-48125) Versus Placebo for the Treatment of Post Traumatic Headache

Primary Endpoint:

The primary endpoint is the mean change from baseline (28-day run-in period) in the number of headache hours of any severity during the 28-day period after the last (3rd) dose of study drug.

Secondary Endpoints:

- Proportion of patients reaching at least 50% reduction in the monthly headache days of any severity during 3 months of treatment with study drug
- Mean change from baseline (28-day run-in period) in the number of headache hours of any severity during the 28-day period after the 1st dose of study drug
- Mean change from baseline (28-day run-in period) in the use of any acute headache medications during the 28-day period after the last (3rd) dose of study drug
- Mean change from baseline (day 0) in disability score, as measured by the 6-item Headache Impact Test (HIT-6) at 28 days after administration of the last (3rd) dose of study drug

Sample Size

150 patients (30 per arm).

Study arms

- Arm 1. Placebo
- Arm 2. TEV-48125 SC 225 mg monthly
- Arm 3. TEV-48125 SC 675 mg monthly
- Arm 4. TEV-48125 IV 675 mg one dose
- Arm 5. TEV-48125 IV 1000 mg one dose

Inclusion criteria

Participants with a history of Chronic Post Traumatic headache as per the ICHD-3.

Exclusion Criteria:

- Current enrollment in or discontinuation within the last 30 days from, a clinical trial involving any investigational drug or device.
- Current use or any prior exposure to any calcitonin-gene-related peptide (CGRP) antibody, any antibody to the CGRP receptor, or antibody to nerve growth factor (NGF).
- Failed more than 4 adequate trials of preventive medication
- Known hypersensitivity to multiple drugs, monoclonal antibodies or other therapeutic proteins.
- A history or presence of other medical illness that indicates a medical problem that would preclude study participation.
- Evidence of significant active or unstable psychiatric disease, in the opinion of the investigator.

- Women who are pregnant or nursing

Table 11: Study Procedures and Assessments

Study period	**Pretreatment period (incl. screening visit and run-in period)**	**Double-blind treatment period**				**Follow-up period**
Visit number	**V1**[a)]	**V2**[b)]	**V3**	**V4**	**V5**[c)]	**V6**[d)]
Month number	**Month -1**	**Month 0**	**Month 1**	**Month 2**	**Month 3**	**Month 9.5**
Procedures and assessments[e)]	**Screening days -28 to -1**	**Baseline dose 1 day 0 (+3 days)**	**Dose 2 day 28 (±3 days)**	**Dose 3 day 56 (±3 days)**	**EOT or early withdrawal day 84 (±3 days)**	**Final visit day 281 (±15 days)**
Informed consent	X					
Medical and psychiatric history	X					
Prior medication history	X					
Inclusion and exclusion criteria	X	X				
Physical examination, including weight and height[f)]	X	X			X	
12-lead ECG[g)]	X	X	X	X	X	
Vital signs measurement[h)]	X	X	X	X	X	
Adverse events[i)]	X	X	X	X	X	X
Concomitant medication inquiry	X	X	X	X	X	X
Clinical laboratory tests[j)]	X	X	X	X	X	
Serum/urine β-HCG test[k)]	X	X			X	
FSH[l)]	X					
Electronic headache diary[m)]	X	X	X	X	X	
Blood samples for plasma drug concentration		X	X	X	X	X
Blood samples for serum ADA concentration		X	X		X	X
Blood sample for pharmacogenomic analysis[n)]		X				

Table 11: Study Procedures and Assessments (Continued)

Study period	**Pretreatment period (incl. screening visit and run-in period)**	**Double-blind treatment period**				**Follow-up period**
Visit number	**V1[a)]**	**V2[b)]**	**V3**	**V4**	**V5[c)]**	**V6[d)]**
Month number	**Month -1**	**Month 0**	**Month 1**	**Month 2**	**Month 3**	**Month 9.5**
Procedures and assessments[e)]	**Screening days -28 to -1**	**Baseline dose 1 day 0 (+3 days)**	**Dose 2 day 28 (±3 days)**	**Dose 3 day 56 (±3 days)**	**EOT or early withdrawal day 84 (±3 days)**	**Final visit day 281 (±15 days)**
Blood collection for further biomarker analysis		X		X	X	
Urine collection for further biomarker analysis		X		X	X	
DISABILITY (TBD)		X			X	
COGNITION (TBD)		X			X	
QOL (TBD)		X			X	
PGIC questionnaire			X	X	X	
Administration of study drug		X	X	X		
Injection site assessments[o)]		X	X	X		

Example 14: Phase 2 Study

A Phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group study comparing the efficacy and safety of two dose regimens of TEV-48125 (one subcutaneous and one intravenous dose regimens) versus placebo for the treatment of persistent post-traumatic headache (PPTH).

Study Population

The study population will be composed of male and female patients, aged 18 to 70 years, inclusive, with a history of Persistent Post-Traumatic Headaches (as defined by

International Classification of Headache Disorders, third revision [ICHD-3] criteria (IHS 2013).

This will include patients with persistent headache attributed to mild traumatic injury of the head and patients with persistent headache attributed to whiplash.

Traumatic injury to the head is defined as a structural or functional injury resulting from the action of external forces on the head. These include striking the head with or the head striking an object, penetration of the head by a foreign body, forces generated from blasts or explosions, and other forces yet to be defined.

The duration of post-traumatic amnesia is defined as the time between head injury and recovery of memory of current events and those occurring in the last 24 hours.

PPTH is a headache attributed to mild head injury with a Glasgow Coma Scale score (GCS) of 13 to 15, loss of consciousness less than 30 minutes and duration of post-traumatic amnesia of less than 24 hours defined as the time between head injury and recovery of memory of current events. Also, two or more other symptoms suggestive of mild traumatic brain injury: nausea, vomiting, visual disturbances, dizziness and/or vertigo, impaired memory and/or concentration.

Persistent headache attributed to whiplash is a headache has developed within 7 days after the whiplash and with greater than three months' duration. Whiplash, associated at the time with neck pain and/or headache. Whiplash is defined as sudden and inadequately restrained acceleration/deceleration movements of the head with flexion/extension of the neck. Whiplash may occur after either high or low impact forces.

Study Endpoints

Primary endpoint: The primary endpoint is the mean change from baseline (28-day run-in period) in the monthly average number of headache days of at least moderate severity during the four weeks period after the administration of study drug.

Secondary endpoints: The secondary endpoints are:

- proportion of patients reaching at least 50% reduction in the monthly average headache days of any severity during the 12 week period of treatment with study drug
- mean change from baseline (28-day run-in period) in the number of headache days of any severity during the 5 to 8 week period after the 1st dose of study drug
- mean change from baseline (28-day run-in period) in the number of headache days of any severity during the 12 week period after the 1st dose of study drug
- mean change from baseline (day 0) in disability score, as measured by the 6-item Headache Impact Test (HIT-6) at 12 weeks after administration of first study drug.

Exploratory Endpoints: The exploratory endpoints are as follows:

- mean change from baseline (28-day run-in period) in the number of headache days of at least moderate severity during the 12 week period after the first dose of study drug
- proportion of patients reaching at least 75% reduction and total (100%) reduction in the monthly average number of headache days of any severity during the 12-week period after the first dose of study drug
- proportion of patients reaching at least 50% reduction, at least 75% reduction, in the number of headache days of any severity during the four week period after the first dose of study drug relative to the baseline period who sustain this level of response over the 12 week period after the first dose of study drug
- mean change from baseline (28-day run-in period) in the monthly average number of headache days of any severity during the 12-week period after the first dose of study drug
- mean change from baseline (28-day run-in period) in the monthly average number of headache days of at least moderate severity during the 4-week period after the first dose of study drug

- proportion of patients reaching at least 50% reduction, at least 75% reduction and total (100%) reduction, in the monthly average number of headache days of at least moderate severity during the eight week period after the first dose of study drug relative to the baseline period
- mean change from baseline (28-day run-in period) only for patients with whiplash, in the monthly average number of neck pain days of any severity during the 12 week period after the first dose of study drug
- mean change from baseline (28-day run-in period) in the use of any acute headache medications (triptans and ergot compounds) during the 12 week period after the administration of first study drug
- mean change from baseline (28-day run-in-period) in the use of opioids, during the 12 week period after the first dose of study drug
- mean change from baseline (day 0) in disability score, as measured by the 6-item Headache Impact Test (HIT-6) at four weeks after administration of first study drug
- mean change from baseline (day 0) in the health status, as measured by the 12-Item Short-Form Health Survey (SF-12) physical and mental health, at four weeks after first dose of study drug administration
- mean change from baseline (day 0) in the assessment of patient satisfaction, as measured by the Patient Global Impression of Change (PGIC) scale, at 4, 8, and 12 weeks after administration of the first dose of study drug
- mean change from baseline (day 0), as measured by the SCAT-3 “Sports concussion assessment tool-3rd” to end of evaluation period week 12 after the first dose of study drug.

General Design and Methodology:

See Table 12 for study procedures and assessments.

A Multicenter, Randomized, Proof of Concept, Double-Blind, Placebo-Controlled, Parallel Group Study Comparing the Efficacy and Safety of two Dose Regimens of TEV-48125 (1 Subcutaneous and 1 intravenous) Administration Versus Placebo with a

diagnosis of persistent post traumatic headache (PTH). The study will consist of a screening visit, a 28-day run-in period, and a double-blind treatment period lasting approximately 12 weeks.

Patients will complete a screening visit (visit 1) after providing written informed consent, and eligible patients will enter a run-in period lasting approximately four weeks (28 days) during which they will enter baseline persistent post-traumatic headache (PPTH) attacks information into an electronic headache diary device daily. Patients will return to the study center after completing the run-in period (visit 2). Patients meeting eligibility requirements at the screening visit and following the 28-day run-in period were randomized to one of three treatment groups:

one treatment group received first dose of TEV-48125 900 mg IV in an infusion during one hour, quarterly;

one treatment group received a first dose of TEV-48125 a 675 mg SC followed by 225 mg SC on the following two months; and

one treatment group received three monthly doses of placebo.

Randomization will be performed using electronic interactive response technology (IRT).

Blinded treatment will be administered IV and SC the first month and SC the second and third month for a total of three doses. First treatment administration will occur at visit 2 and additional doses will be administered at visits 3 and 4. Patients will return to the study center approximately every four weeks for blinded treatment administered SC for safety and efficacy assessments and blood and urine sampling for pharmacokinetic, immunogenicity, biomarkers, and pharmacogenomics (unless prohibited by local regulations) analyses. Final study assessments will be performed at the final visit for this study (visit 5), approximately 12 weeks after administration of study drug.

Method of Blinding and Randomization:

The sponsor, investigators, study staff (except for staff involved in bioanalytical analyses) and patients will be blinded to treatment assignment. A computer-generated master randomization list will be provided to drug packaging facilities. Packaging vendor(s) will package active and placebo into single-visit kits according to Good Manufacturing Practice procedures. Kits will be identical in appearance and contain one vial with active drug or placebo and prefilled syringes (active or placebo). Adequate kit supply for upcoming study visits will be managed by IRT and kept (refrigerated at 2°C to 8°C) on site.

At the end of the screening period, patients will be randomized if they have at least 6 or more headache days of at least moderate severity, during the run in period and at least 85% of diary compliance.

This study is a randomized study with stratification based on gender, the severity of traumatic injury to the head (mild, moderate or severe) according to the IHS Classification. Each patient will undergo randomization in a 1:1:1 ratio within the stratum to which he or she belongs to receive TEV-48125 or placebo, as assigned by the IRT. The IRT will manage initial drug supply, maintenance of adequate study drug supplies on site, and study randomization centrally.

Study Drug Dose, Mode of Administration, and Administration Rate:

Prefilled vials (active or placebo) will be contained in uniquely numbered kits and stored (refrigerated at 2°C to 8°C) on site. Active vials for IV administration (10 mL) will contain TEV-48125 at a concentration of 150 mg/mL, and placebo vials (10 mL) will contain the same vehicle and excipients as those for active infusion and injection. Active syringes will contain 150 mg/mL of TEV-48125 and placebo syringes will contain the same vehicle and excipients as those for active injections. Prefilled syringes (active or placebo) and one vial will be contained in uniquely numbered kits.

Study drug will be administered by qualified study personnel and will retrieve the appropriately numbered kit and extract a volume of the vial contents and add it to 500 mL of normal saline solution, or a SC injections and administered as follows:

- Patients randomized to receive TEV-48125 will receive 900 mg of TEV-48125 as a 1-hour IV infusion and 3(three) 1.5 mL placebo injections SC at visit 2, and single 1.5 mL, placebo injections SC at visits 3 and 4.
- Patients randomized to receive TEV 41825 will receive 675 mg as three active injections (225 mg/1.5 mL) SC and placebo as a 1-hour IV infusion at visit 2, and single 1.5 mL, 225 mg active injections SC at visits 3 and 4.

Patients randomized to receive placebo will receive placebo as a 1-hour IV infusion and three 1.5 mL placebo injections SC at visit 2, and single 1.5 mL placebo injection SC at visits 3 and 4.

Investigational Product: TEV-48125
Placebo: Same vehicle and excipients as those for active

Duration of Patient Participation: Patient participation will last for approximately 16 weeks (including a run-in period lasting approximately four week and a 12 week double-blind treatment period). Patients are expected to complete the entire duration of the study.

Criteria for Inclusion: Patients may be included in the study if they meet all of the following criteria:

a. Participants with a diagnosis of Persistent Post Traumatic headache as per the ICHD-3 (beta version) criteria
b. Traumatic injury to the head has occurred defined as a structural or functional injury resulting from the action of external forces on the head. These include striking the head with or the head striking an object, penetration of the head by a foreign body, forces generated from blasts or explosions, and other forces yet to be defined.
c. Headache is reported to have developed within seven days after one of the following:
    1. mild traumatic injury to the head
    2. regaining of consciousness following the injury to the head

3. discontinuation of medication(s) that impair ability to sense or report headache following the injury to the head

d. Headache persists for greater than 3 months after the injury to the head Persistent post-traumatic headache (PPTH) attributed to whiplash has developed within 7 days after the whiplash and with greater than 3 months' duration.

There will be no more than 30% of patients with PPTH with whiplash with no evidence of head injury.

e. The patient signs and dates the informed consent document.

f. Males or females aged 18 to 70 years, inclusive

g. The patient has at least six or more headache days of at least moderate severity during the run-in period.

h. The patient is in good health as determined by a medical history, medical examination, ECG, serum chemistry, hematology, urinalysis, and serology.

i. All subjects must be of non-childbearing potential, defined as:

- women surgically sterile by documented complete hysterectomy, bilateral oophorectomy, or bitubal ligations or confirmed to be postmenopausal (at least one year since last menses and follicle stimulating hormone [FSH] above 35 U/L);
- men surgically sterile by documented vasectomy; or
- if of childbearing potential, subjects must meet any of the following criteria:
  - Subjects must simultaneously use two forms of highly effective contraception methods with their partners during the entire study period and for 7.5 months after the last dose of study drug.
  - Sexual abstinence is only considered a highly effective method if defined as refraining from heterosexual intercourse in the defined period. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical study and the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of

abstinence for the duration of a study, and withdrawal are not acceptable methods of contraception.

j. Female subjects of childbearing potential must have negative serum beta human chorionic gonadotropin (β-HCG) pregnancy test at screening (confirmed by serum β-HCG pregnancy test at check-in).

k. The patient, if a man, is surgically sterile, or, if capable of producing offspring, has exclusively same-sex partners or is currently using an approved method of birth control and agrees to continued use of this method for the duration of the study. Acceptable methods of contraception include abstinence, female partner's use of steroidal contraceptive (oral, implanted or injected) in conjunction with a barrier method, female partner's use of an intrauterine device, or if female partner is surgically sterile. In addition, male patients may not donate sperm for the duration of the study.

l. The patient must be willing and able to comply with study restrictions and to remain at the clinic for the required duration during the study period, and willing to return to the clinic for the follow-up evaluation as specified in this protocol.

Criteria for Exclusion: Patients will be excluded from participating in this study if they meet any of the following criteria:

a. Previous history of brain imaging showing evidence of intracranial hemorrhage, subdural or epidural hematomas and subarachnoid hemorrhages as a consequence of the traumatic head injury.

b. PPTH attributed to craniotomy

c. Patient has another headache disorder not attributable to head trauma

d. Patients with previous history (before the headache trauma) of any type of headache that has >6 headache days per month

e. Patient is using medications containing opioids (including codeine) and barbiturates containing analgesics on average more than 10 days per month.

f. Current enrollment or previous participation, within the last 30 days from, in a clinical trial involving any investigational drug or device, or 5.5 half-lives, whatever is longer

g. Current use or any prior exposure to any calcitonin-gene-related peptide (CGRP) antibody, any antibody to the CGRP receptor, or antibody to nerve growth factor (NGF).

h. Known hypersensitivity to multiple drugs, monoclonal antibodies or other therapeutic proteins.

i. A history or presence of other medical illness that indicates a medical problem that would preclude study participation.

j. Evidence of significant active or unstable psychiatric disease, in the opinion of the investigator.

k. The patient is a pregnant or lactating woman. (Any woman becoming pregnant during the study will be withdrawn from the study.)

l. The patient has previously participated in a Teva sponsored clinical study with study drug.

m. Clinically significant hematological, cardiac, renal, endocrine, pulmonary, gastrointestinal, genitourinary, neurologic, hepatic, or ocular disease, and patient has any clinically significant uncontrolled medical condition (treated or untreated) at the discretion of the investigator.

n. The patient cannot participate or successfully complete the study, in the opinion of their healthcare provider or the investigator, for any of the following reasons:

- mentally or legally incapacitated or unable to give consent for any reason
- in custody due to an administrative or a legal decision, under tutelage, or being admitted to a sanitarium or social institution
- unable to be contacted in case of emergency
- has any other condition, which, in the opinion of the investigator, makes the patient inappropriate for inclusion in the study

Measures and Time Points:

Primary Efficacy Measure and Time Point: The primary efficacy endpoint for this study will be derived from persistent post-traumatic headache days data (i.e., occurrence of headaches days, duration of the headaches, severity of headaches, and acute headache-specific medication use) collected daily using an electronic headache diary device.

Eligible patients will receive training on the use of the electronic headache diary device and will be informed of compliance requirements at screening. Patients will complete electronic headache diary entries with questions about the previous day daily, beginning on the day after the screening visit through the EOT/early withdrawal visit. The electronic headache diary device will allow entry of headache information for up to 24 hours after a given day.

Secondary Efficacy Measures and Time Points: Secondary efficacy endpoints will be derived from headache days data (i.e., occurrence, duration of headache, severity of headaches, and acute headache-specific medication use collected daily using an electronic headache diary device. In addition, patient perception of improvement will be evaluated by the Headache Impact Test (HIT-6) after 8 weeks of first dose of study drug administration

Exploratory Efficacy Measures and Time Points: The following exploratory efficacy measures will be assessed:

- exploratory efficacy endpoints derived from PTH days data (i.e., occurrence headache days, duration, severity of headache, and acute headache-specific medication use, which are collected daily using an electronic headache diary device
- Headache Impact Test (HIT-6 questionnaire)
- SF-12 physical and mental health
- Patient Global Impression of Change (PGIC) questionnaire
- SCAT-3 "Sports concussion assessment tool-3rd

Safety Measures and Time Points:

Safety and tolerability will be assessed using the following measures:

- inquiries about adverse events
- inquiries about concomitant medication usage
- safety laboratory tests (serum chemistry, hematology, coagulation, and urinalysis)
- serum/urine β-HCG test (women of childbearing potential only)
- 12-lead ECGs
- vital signs measurements (systolic and diastolic blood pressure, pulse, and oral temperature. Oxygen saturation should be measured in cases of suspected anaphylaxis. Respiratory rate will also be measured in these cases (but not as a standard vital sign)
- physical examinations, including body weight
- hypersensitivity reaction assessment
- eC-SSRS

Pharmacokinetics/Biomarkers/Immunogenicity and Biomarker Substudy Measures and Time Points: Pharmacokinetic Measures and Time Points: Blood samples for pharmacokinetics analysis of TEV-48125 will be collected from all patients for the purpose of a population pharmacokinetic modeling approach and pharmacokinetic/pharmacodynamic relationship assessment. The pharmacodynamic parameters will be the efficacy responses.

The actual date and time of each blood sample, as well as the date and time of dosing prior to each sample, will be recorded in the case report form. TEV-48125 plasma concentration will be measured using a validated assay.

Immunogenicity Measures and Time Points: Blood samples for immunogenicity will be collected.

Biomarker Measures and Time Points: Biomarker blood and urine samples will be collected from all patients, and a blood sample will be collected from patients at visit 2 or at any visit thereafter (unless prohibited by local regulation).

Allowed and Disallowed Medications Before and During the Study:

No more than 2 medications regardless of the indication and no restrictions in abortive agents except opioids and barbiturates (refer to exclusion criteria)

Statistical Considerations:

Sample Size Rationale: No prospective calculations of statistical power have been made. A sample size of 75 patients (25 patients per treatment arm group) is chosen based on clinical and practical considerations.

Analysis of Primary Endpoint: The primary efficacy endpoint, the mean change from baseline (28-day run-in period) in the monthly average number of headache days of at least moderate severity during the four weeks period after the administration of study drug, will be analyzed using an analysis of covariance (ANCOVA) method. The model will include treatment, gender, and the severity (mild, moderate, or severe) of traumatic injury to the head as fixed effects. Ninety five percent confidence intervals will be constructed for the least squares mean differences between each TEV-48125 group and the placebo group.

Analysis of Secondary and Exploratory Endpoints: The same analysis used for the primary efficacy endpoint will be performed for the continuous secondary and exploratory efficacy endpoints. For the proportion of responders defined as 50% or more reduction from baseline in the monthly average headache days, Cochran-Mantel-Haenszel test will be used.

Multiple Comparisons and Multiplicity: There will be no multiple comparisons.

Safety Analyses:

All adverse events will be coded using the Medical Dictionary for Regulatory Activities. Each patient will be counted only once in each preferred term or system organ class category for the analyses of safety. Summaries will be

presented for all adverse events (overall and by severity), adverse events determined by the investigator to be related to study drug (defined as related or with missing relationship) (overall and by severity), serious adverse events, and adverse events causing withdrawal from the study. Patient listings of serious adverse events and adverse events leading to withdrawal will be presented.

Local tolerability findings will be listed and summarized descriptively.

Changes in laboratory and vital signs measurement data will be summarized descriptively. All values will be compared with pre-specified boundaries to identify potentially clinically significant changes or values, and such values will be listed.

The use of concomitant medications will be summarized by therapeutic class using descriptive statistics. Concomitant medications will include all medications taken while the patient is treated with study drug.

Safety data will be summarized descriptively overall and by treatment group. For continuous variables, descriptive statistics (n, mean, SD, median, minimum, and maximum) will be provided for actual values and changes from baseline to each time point. For categorical variables, patient counts and percentages will be provided. Descriptive summaries of serious adverse events, patient withdrawals due to adverse events, and potentially clinically significant abnormal values (clinical laboratory or vital signs) based on predefined criteria will also be provided.

If any patient dies during the study, a listing of deaths will be provided, and all relevant information will be discussed in the patient narrative included in the clinical study report.

Immunogenicity Analysis: Summary of immunogenicity results will be provided, and the incidence of immunogenicity will be calculated. The impact of

immunogenicity on the pharmacokinetic profile, drug efficacy, and clinical safety will be evaluated. This analysis will be reported separately.

Biomarker Analysis: Biomarker analysis will include logistic regression, receiver operating characteristic curves, and summary statistics. Results will be reported separately. Measurements will be made using validated assays.

Table 12: Study Procedures and Assessments

Study period	**Pretreatment period (incl. screening visit and run-in period)**	**Double-blind treatment period**				**Follow-up period**
Visit number	**V1**[a)]	**V2**[b)]	**V3**	**V4**	**V5**[c)]	**V6**[d)]
Month number	**Month -1**	**Month 0**	**Month 1**	**Month 2**	**Month 3**	**Month 9.5**
Procedures and assessments[e)]	**Screening days -28 to -1**	**Baseline dose 1 day 0 (+3 days)**	**Dose 2 day 28 (±3 days)**	**Dose 3 day 56 (±3 days)**	**EOT or early withdrawal day 84 (±3 days)**	**Final visit day 281 (±15 days)**
Informed consent	X					
Medical and psychiatric history	X					
Prior medication history	X					
Inclusion and exclusion criteria	X	X				
Physical examination, including weight and height[f)]	X	X			X	
12-lead ECG[g)]	X	X	X	X	X	
Vital signs measurement[h)]	X	X	X	X	X	
Adverse events[i)]	X	X	X	X	X	X
Concomitant medication inquiry	X	X	X	X	X	X
Clinical laboratory tests[j)]	X	X	X	X	X	
Serum/urine β-HCG test[k)]	X	X			X	
FSH[l)]	X					
Electronic headache diary[m)]	X	X	X	X	X	
Blood samples for plasma drug concentration		X	X	X	X	X
Blood samples for serum ADA		X	X		X	X

Study period	Pretreatment period (incl. screening visit and run-in period)	Double-blind treatment period				Follow-up period
Visit number	**V1[a)]**	**V2[b)]**	**V3**	**V4**	**V5[c)]**	**V6[d)]**
Month number	**Month -1**	**Month 0**	**Month 1**	**Month 2**	**Month 3**	**Month 9.5**
Procedures and assessments[e)]	**Screening days -28 to -1**	**Baseline dose 1 day 0 (+3 days)**	**Dose 2 day 28 (±3 days)**	**Dose 3 day 56 (±3 days)**	**EOT or early withdrawal day 84 (±3 days)**	**Final visit day 281 (±15 days)**
concentration						
Blood sample for pharmacogenomic analysis[n)]		X				

Table 12: Study Procedures and Assessments (Continued)

Study period	Pretreatment period (incl. screening visit and run-in period)	Double-blind treatment period				Follow-up period
Visit number	**V1[a)]**	**V2[b)]**	**V3**	**V4**	**V5[c)]**	**V6[d)]**
Month number	**Month -1**	**Month 0**	**Month 1**	**Month 2**	**Month 3**	**Month 9.5**
Procedures and assessments[e)]	**Screening days -28 to -1**	**Baseline dose 1 day 0 (+3 days)**	**Dose 2 day 28 (±3 days)**	**Dose 3 day 56 (±3 days)**	**EOT or early withdrawal day 84 (±3 days)**	**Final visit day 281 (±15 days)**
Blood collection for further biomarker analysis		X		X	X	
Urine collection for further biomarker analysis		X		X	X	
HIT 6 questionnaire		X			X	
		X			X	
SF 12 questionnaire		X			X	
PGIC questionnaire			X	X	X	
Administration of study drug		X	X	X		
Injection site assessments[o)]		X	X	X		

Example 15: Development of Headache-Related Behaviors in a Clinically Relevant Rat Model of Posttraumatic Headache are mediated by CGRP

Materials and Methods

Animals

Male Sprague-Dawley rats (Taconic, USA) weighing 250-300g at time of arrival were used in all studies. Animals were housed in pairs under a constant 12 hour light/dark (lights on at 07:00h) cycle at room temperature. Food and water were available ad libitum. In all experiments animals were randomly assigned to either sham or mild closed head injury (mCHI) groups as well as for the different treatments and group were tested in a blinded fashion. All experiments were approved and conducted in compliance with the institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Centre and Harvard Medical School and were in compliance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

Experimental Mild Closed Head Injury

Experimental mCHI was induced using the weight-drop concussive device as described previously (Marmarou et al., J of Neurosurgery 1994;80(2):291-300; Mychasiuk et al., J Neurotrauma 2014;31(8):749-757). Briefly, rats were anesthetized with 3% isoflurane and placed chest down directly under a weight-drop concussive head trauma device. The device consisted of a hollow cylindrical tube (inner diameter 2.54 cm) placed vertically over the rat's head. To induce a head trauma, a 250 g weight was dropped through the tube from a height of 80 cm, striking the center of the head. A foam sponge (thickness 3.81 cm, density 1.1 g/cm^3) was placed under the animals to support the head while allowing some anterior-posterior motion without any rotational movement at the moment of impact. Immediately after the impact animals were returned to their home cages for recovery. All animals regained consciousness within 2 minutes of injury and were neurologically assessed in the early hours and days post-injury for any behavioral abnormalities suggestive of neurological impairment. Sham animals were anesthetized but not subjected to the weight drop. All animals subjected to PTH behavioral assessments did not display any major neurological deficits.

Behavioral Testing

Open Field Monitoring System

Activity Monitor SOF-811 (Med Associates, Vermont, USA) was used to measure locomotor activity in an open field environment. The system consists of a two areas setup and evaluates the movement of animals in the horizontal (X-Y axis) and vertical (Z-axis) planes. Each plane was monitored by 16 beams spaced 2.54 cm apart, and there was an infrared emitter and detector per beam located on each side of each arena to monitor movement across the width of the arena. Data were sent from the 3 sets of detector-emitters to a central computer displaying a range of pre-selected outputs including total distance moved and vertical activity (rearing) during 60 second intervals and were analysed as a total over 20 minutes. Each arena was lit with a single white LED bulb on a dimmer switch to maintain a homogenous lighting across the arenas (80 lux). The arenas were cleaned with mild detergent and dried to remove odour cues between successive rats. Testing was conducted at baseline (24 hours prior to head trauma) and then at 48 hours, 72 hours, 7 and 14 days post mCHI.

Novel Object Recognition

The novel object recognition test is designed to evaluate deficits in recognition memory in rodents, as a measure of the severity of the brain injury. The procedure used for the novel-object recognition test was similar to that described previously (King et al., Neuropharmacology 2004;47(2):195-204; Moriarty et al., Behavioural brain research 2016;303:61-70), with some modifications. The apparatus was constructed of Perspex arena (45 cm^2). The "familiar" objects used were two identical shapes composed of "Lego DUPLO" building blocks. A third object, the "novel object" consisted of a plastic bottle covered with green tape. Animals were habituated to the arena in the absence of objects for 30 minutes on the day before the test day. The test day comprised three stages: habituation (5 minute exposure to arena in absence of objects), exposure 1 (5 minute familiarisation to identical objects) and exposure 2 (5 minute exposure to one familiar and one novel object). Habituation and exposure 1 were separated by a 5 minute inter-trial interval and exposures 1 and 2 by a further 5 minute

interval. Exploration of an object was defined as any behaviour directed toward it (i.e., sniffing, rearing, leaning or climbing). The three test stages were recorded for subsequent analysis and object exploration was manually rated in a blind manner. The discrimination ratio for each object was calculated as time spent exploring either object/time spent exploring both objects. A Preference Index above 0.5 (i.e., 50%) indicates novel object preference, below 0.5-familiar object preference and 0.5 itself- no preference.

Von Frey Testing

The method used was previously used to study headache-related behaviours (Edelmayer et al., Methods Mol Biol 2012;851:109-120; Oshinsky, Headache 2007;47(7):1026-1036; Yan et al., Mol Pain 2012;8:6, Zhao et al., Pain 2014;155(7):1392-1400). Briefly, animals were placed in a transparent flat-bottomed acrylic holding apparatus (20.4cm x 8.5cm). The apparatus was large enough to enable to the animals to escape the stimulus. Animals were habituated to the arena for 15 minutes prior to testing. In order to determine if animals developed pericranial mechanical hypersensitivity following mCHI the skin region, including the midline area above the eyes and 2 cm posterior, was stimulated with different von Frey filaments (0.6g-10g) (18011 Semmes-Weinstein Anesthesiometer Kit). Development of hindpaw hypersensitivity was tested by stimulating the mid-dorsal part of the hindpaw. Changes in tactile skin sensitivity were evaluated similar to previous studies (Levy et al., Brain, Behavior, and Immunity 2012;26(2):311-317) by recording four behavioural responses adapted from Vos et al. (Journal of Neuroscience: the official journal of the Society for Neuroscience 1994;14(5 Pt 1):2708-2723) as follows: 0) No response: rat did not display any response to stimulation 1) Detection: rat turned its head towards stimulating object and latter is explored usually by sniffing; 2) Withdrawal: rat turned its head away or pulled it briskly away from stimulating object (usually followed by scratching or grooming of stimulated region); 3) Escape/Attack: rat turned its body briskly in the holding apparatus in order to escape stimulation or attacked (biting and grabbing movements) the stimulating object. Starting with the lowest weight, each filament was applied three times with an intra-application interval of 5 seconds and the behaviour that

was observed at least twice was recorded. For statistical analysis the score recorded was based on the most aversive behaviour noted. The force that elicited three consecutive withdrawal responses was considered the response threshold. To evaluate pain behaviour in addition to changes in threshold, for each rat, at each time point, a cumulative response score was determined by combining the individual scores (0–3) for each one of the VF filaments tested. All tests were conducted and evaluated in a blinded manner. Responses to von Frey stimuli were tested at baseline and also 48 hours, 72 hours as well at 7 and 14 days post mCHI.

Conditioned Place Preference using sumatriptan

The method employed was adapted from previously published reports (Griggs et al., Neuroreport 2015;26(9):522-527). Two CPP boxes (Med Associates, Vermont, USA), consisting of two separate chambers, were used to assess chamber preference before and after the drug-conditioning phase. Sumatriptan was chosen for the initial characterization experiment as it has previously demonstrated efficacy with CPP in a pre-clinical migraine model (De Felice et al., Annals of neurology 2013;74(2):257-265). On the pre-conditioning day (Day 6 post mCHI) sham or mCHI rats were placed into CPP boxes with access to both chambers for a period of 20 minutes and time spent in each chamber was recorded. Chambers were discriminated between based on visual (walls) and tactile (floors) cues. On the morning of the conditioning day (Day 7 post mCHI), animals received a vehicle control (saline i.p.), and 2 hours later were confined to the vehicle-paired chamber for 20 minutes. In the afternoon, 4 hours after vehicle injection, animals received sumatriptan, and 2 hours later were confined to the opposite chamber (sumatriptan-paired chamber) for 20 minutes. A 2 hour period was chosen between administration of sumatriptan and chamber confinement as data suggests that optimal efficacy of sumatriptan is observed 2 hours post administration (Tfelt-Hansen et al., Headache 1998;38(10):748-755). 24 hours later (Day 8 following mCHI) animals were placed into the CPP apparatus with free access to both chambers for 15 minutes and time spent in each chamber was recorded.

GTN-evoked mechanical hypersensitivity

Low dose GTN (100 µg/kg) was administered on Day 15 and 30 post mCHI to investigate if any increased susceptibility to common headache triggers existed in mCHI animals following resolution of the cephalic pain hypersensitivity 2 weeks post mCHI. Development of pericranial and hindpaw mechanical hypersensitivity was assessed as above at 1 hour and 4 hours post-GTN administration. Acute administration of sumatriptan or chronic blockade of CGRP action using a murine mAb were investigated for their ability to attenuate the GTN-induced pain hypersensitivity.

GTN induced conditioned place aversion

Conditioned place aversion to GTN was tested using the two CPP boxes as indicated above. The protocol included a pre-condition day (Day 13 post mCHI), followed by a conditioning day (Day 14) and a post conditioning day 24 hours later. On the morning of the conditioning day, animals were first confined for 20 minutes to one chamber, prior to GTN administration (pre-GTN-paired chamber). Animals were then administered with GTN and 4 hours later were confined to the opposite chamber (GTN-paired chamber) for 20 minutes. On the post-conditioning day animals had again free access to both chambers. Aversion to GTN was determined by calculating difference scores between the times spent in the different chambers during the pre-conditioning and post-conditioning days in animals treated with the murine anti-CGRP mAb or the control IgG.

Drugs

Sumatriptan (Sigma, USA) was freshly dissolved in 0.9% saline and administered intra-peritoneal (i.p.) at a dose of 1mg/kg in a volume of 1 ml/kg. All behavioural testing was conducted 2 hours after sumatriptan administration. Drug dose and times of administration were based on the pharmacokinetics of the drugs as well as in-house pilot work and published studies demonstrating their efficacy in animal models of trigeminal pain (Oshinsky et al., Headache 2012;52(9):1336-1349; Winner et al., Mayo Clin Proc 2003;78(10):1214-1222). A murine-specific mAb targeting CGRP and its control IgG were provided by Teva Pharmaceuticals and were administered i.p. at a dose of 30 mg/kg in a volume of 0.54 ml/100g (Kopruszinski et al., Cephalalgia: an

International Journal of Headache 2016). The first administration was delivered immediately after mCHI induction and every 6 days subsequently. GTN (American Reagent, USA) was freshly dissolved in 0.9% saline and administered intra-peritoneal (i.p.) at a dose of 100 µg/kg in a volume of 1 ml/kg. The final vehicle concentration for the GTN was 0.6% propylene glycol, 0.6% ethanol and 0.9% saline. Previous work has shown no effect on mechanical thresholds with 6% propylene glycol and ethanol vehicle concentration (Pradhan et al., Pain 2014;155(2):269-274).

Data Analysis

Statistical analyses were conducted using Statview (SAS Institute, New York, NY, USA). Normality and homogeneity of variance were assessed using Shapiro-Wilk and Levene tests, respectively. Two-way repeated measures analysis of variance (ANOVA) was performed to determine main effects of time, head trauma, and drug treatment, or their interaction, on locomotor activity in the open field or the development of pericranial hypersensitivity using the von Frey apparatus. Fisher's LSD post hoc test or Student's unpaired 2-tailed t-tests were used, as appropriate, to assess differences between the groups and across time points. Data are presented as mean ± standard error of the mean. $p<0.05$ was considered statistically significant.

Results

Reduced vertical rearing activity but no evidence of cognitive deficits in mCHI/concussed animals

Following mCHI, animals displayed reduced vertical exploratory (rearing) activity in the open field (RM ANOVA time: $F_{(4, 56)}=7.03$, $p<0.01$, injury $F_{(1, 14)}=21.31$, $p<0.01$). Student's unpaired two-tailed t-tests revealed that the mCHI group displayed consistently decreased vertical exploratory activity compared to the sham group at 48 hours, 72 hours, and Day 7 post mCHI ($p<0.01$) (Figure 12A), indicating a mild traumatic brain injury. At these time points, however, no changes were detected in total distance travelled, centre zone exploration or thigmotaxis (all $p>0.05$). There was also no evidence of major cognitive deficits in mCHI animals at 7 days post-injury as measured by the novel object recognition test ($p>0.05$) (Figure 12B).

Development of tactile hypersensitivity in mCHI animals

Using von Frey filaments, a time dependent development of cephalic tactile hypersensitivity was identified in mCHI, but not in sham animals. Repeated measures ANOVA revealed a significant effect of time ($F_{(4, 48)}=7.54$, $p<0.001$) and a time x injury interaction ($F_{(4,48)}=5.71$, $p<0.001$). Student's unpaired two-tailed t-tests revealed that response thresholds were significantly reduced and nociceptive scores in the mCHI group were significantly increased at 72 hours and Day 7 (both $p<0.05$) compared to the sham group (Figures 13A and 13C). At any of the time point tested, mCHI did not lead to development of hindpaw mechanical hypersensitivity (Figures 13B and 13D).

Effects of acute sumatriptan treatment and chronic CGRP blockade on mCHI-induced cephalic tactile hypersensitivity

Overall, acute sumatriptan treatment at 72h post-CHI attenuated cephalic tactile hypersensitivity. (One-way ANOVA $F_{(2, 17)}=8.79$, $p<0.05$) (Figures 14A and 14B). The ability of chronic blockade of CGRP using injections of a blocking mAb, or control IgG, starting immediately after mCHI and every 6 days subsequently was tested on the development of mCHI-related cephalic tactile hypersensitivity. Overall, CGRP blockade attenuated mCHI-induced mechanical hypersensitivity (RM ANOVA time: $F_{(3, 39)}=17.76$, $p<0.001$). Student's unpaired two-tailed t-tests revealed that anti-CGRP mAb treated group had a significantly increased response threshold and reduced nociceptive score compared to the control IgG-treated group at Day 7 post mCHI (Figures 14C and 14D, $p<0.05$). Chronic treatment with the anti-CGRP mAb did not have any effect on mechanical response threshold in sham animals in agreement with the current finding of Kopruszinski et al., who also employed an anti-CGRP mAb (Kopruszinski et al., Cephalalgia: an International Journal of Headache 2016).

Sumatriptan related conditioned Place Preference in mCHI animals

To investigate the aversive nature of PTH pain in mCHI animals, the CPP paradigm was used to determine whether the anti-migraine drug sumatriptan could produce CPP in mCHI rats but not in sham controls at 7 days post-head injury. Difference scores (postconditioning - preconditioning time difference for each chamber) for individual rats indicate that mCHI rats displayed significantly increased time ($p<0.01$)

in the sumatriptan chamber compared to sham controls (Figure 15B), suggesting that systemic sumatriptan treatment alleviated the aversive, headache-like effect of mCHI.

Development of mechanical hypersensitivity following administration of GTN to asymptomatic mCHI animals

By Day 14 post-mCHI, no significant differences in cephalic tactile sensitivity existed between sham and mCHI groups. To determine if rats subjected to mCHI developed enhanced tactile pain sensitivity to a migraine-triggering event, following the resolution of the acute behavioural symptoms, we tested changes in cephalic and extra-cephalic responses to mechanical stimulation following the administration of the common migraine trigger GTN on Days 15 and 30 post-injury. On Day 15, administration of GTN resulted in renewed and pronounced cephalic mechanical hypersensitivity in mCHI animals (RM ANOVA time: $F_{(1, 14)}=38.68$, $p<0.001$, time x GTN treatment $F_{(2, 14)}=5.18$, $p<0.05$), but not in sham animals. Fisher's LSD post-hoc tests revealed that the mCHI group displayed a significantly reduced cephalic response threshold and increased nociceptive score at 1h ($p<0.01$) and 4h ($p<0.001$) post GTN and compared to sham controls (Figures 16A and 16B). On Day 30 post mCHI, GTN also produced pronounced cephalic hypersensitivity (RM ANOVA treatment: $F_{(1, 13)}=9.7$, $p<0.05$, time x GTN treatment: $F_{(2, 26)}=3.24$, $p<0.05$). Fisher's LSD post-hoc tests revealed that the mCHI group displayed a significantly reduced cephalic response threshold and at 1 h and 4 h post-GTN ($p<0.05$,) compared to sham controls and an increased nociceptive score at 4h post-GTN (Figures 16C and 16D, $p<0.05$).

GTN administration also induced hindpaw mechanical hypersensitivity that was however limited to reduced mechanical thresholds, selectively in mCHI animals on Day 15 (RM ANOVA time: $F_{(2, 26)}=8.27$, $p<0.01$, and GTN treatment: $F_{(1, 14)}=8.12$, $p<0.05$) and Day 30 (RM ANOVA time: $F_{(2, 26)}=37.04$, $p<0.05$, and GTN treatment: $F_{(2, 26)}=3.24$, $p<0.05$) post-injury. Fisher's LSD post-hoc tests revealed that the mCHI group displayed a significantly reduced mechanical thresholds at 4 h post GTN ($p<0.05$) on Day 15 and at 1 h post GTN on Day 30, compared to sham controls (Figures 17A and 17C, $p<0.05$).

Effects of acute sumatriptan treatment and chronic CGRP blockade on GTN-evoked mechanical hypersensitivity in mCHI animals

On day 15 post mCHI, acute sumatriptan treatment attenuated the GTN-evoked delayed mechanical cephalic hypersensitivity (at 4 h) when administered 1 h after GTN. Student's unpaired two-tailed t-tests revealed that acute sumatriptan treatment significantly reduced both the decrease in response threshold, and the increase in nociceptive score compared to vehicle treated animals ($p<0.001$ and $p<0.05$, Figures 18A and 18B). Sumatriptan treatment did not attenuate the GTN-evoked hindpaw hypersensitivity in mCHI animals. Chronic treatment with the anti-CGRP mAb prevented GTN-induced mechanical hypersensitivity on Day 15 post-mCHI. Student's unpaired two-tailed t-tests revealed that the anti-CGRP mAb group displayed a significantly increased response threshold and a reduced nociceptive score compared to the control IgG group ($p<0.05$, $p<0.01$ respectively, Figures 18C and 18D). When compared to treatment with the control IgG, anti-CGRP mAb treatment was also effective at attenuating the GTN-induced reduced mechanical thresholds at 30 days post-injury ($p<0.05$, Student's unpaired two-tailed t-tests). Anti-CGRP mAb treatment, however, was ineffective in blocking the GTN-evoked hindpaw hypersensitivity in mCHI animals.

Conditioned Place Aversion to GTN

Using the conditioned place aversion paradigm, GTN administration following mCHI was tested to see if it could induce ongoing pain-like behaviour that is responsive to anti-CGRP mAb treatments. Administration of GTN to mCHI animals on Day 14 post mCHI resulted in a conditioned place aversion, that is reduced time in the GTN-paired chamber, in animals treated with the control IgG, suggesting GTN-evoked pain in mCHI animals at a times when the cephalic pain hypersensitivity was already resolved. There was no evidence of a similar GTN-evoked conditioned place aversion in mCHI animals treated with the anti-CGRP mAb (Figures 19A and 19B), suggesting that GTN-evoked pain in mCHI animals is CGRP-dependent.

Effect of chronic treatment with anti-CGRP mAb on PTH-related behaviours

The main findings of the study are: 1) Concussion via mCHI was associated with an acute phase of reduced rearing (i.e., exploratory activity), but not with major motor or cognitive deficits, suggesting a mild transient form of traumatic brain injury (Kilbourne et al., J Neurotrauma 2009;26(12):2233-2243), 2) Concussed animals developed cephalic tactile pain hypersensitivity that was resolved by 2 weeks post-injury, and which was attenuated by acute treatment with sumatriptan and prolonged inhibition of CGRP via a blocking mAb, 3) Animals subjected to mCHI displayed spontaneous/ongoing pain-like behaviour in the CPP paradigm when using systemic sumatriptan treatment as an analgesic treatment, and 4) Following resolution of the headache/pain behaviours, administration of the headache trigger GTN resulted in a renewed and pronounced cephalic hypersensitivity as well as conditioned place aversion that was inhibited by sumatriptan and anti-CGRP mAb treatments.

Currently, there are no evidence-based treatment guidelines for PTH management, thus it is often treated similar to the primary headache disorder it resembles, with studies showing the efficacy of sumatriptan in some PTH patients with migrainous features (Abend et al., J Child Neurol 2008;23(4):438-440; Lucas, Curr Pain Headache Rep 2015;19(10):48). However, because a significant proportion of patients do not respond to triptan treatment (Visser et al., Headache 1996;36(8):471-475), other therapy options are needed. Based on the hypothesized role of CGRP in migraine and headache (Pietrobon et al., Annu Rev Physiol 2013;75:365-391; Russo, Annual review of pharmacology and toxicology 2015;55:533-552), a promising emerging therapy strategy targets CGRP or its receptor using blocking mAbs (Mitsikostas et al., BMC Med 2015;13:279). Phase IIb trials of one such mAb, TEV-48125, have demonstrated favourable results in terms of safety, efficacy and tolerability of this agent (Bigal et al., Lancet Neurol 2015;14(11):1081-1090; Bigal et al., Lancet Neurol 2015;14(11):1091-1100). The results from the current study indicate that inhibiting CGRP via a rodent-specific neutralising mAb can reduce the headache/pain symptoms evoked by mCHI suggesting the involvement of CGRP in mediating the development of PTH pain. Treatment with the anti-CGRP mAb was also effective in attenuating the GTN-related

cephalic mechanical hypersensitivity in mCHI animals and attenuated conditioned place aversion to GTN, pointing to CGRP also as a mediator of the hyperalgesic priming-like effect evoked by the head trauma, in particular the headache-like allodynia and ongoing pain induced by GTN. As the mAb is unlikely to cross the blood-brain-barrier (due to its large molecular weight), its mechanism of action in mCHI animals likely involves a peripheral site, potentially the interruption of mCHI-evoked meningeal or periosteal inflammatory response. Because the anti-CGRP mAb treatment was ineffective in ameliorating the development of GTN-evoked hindpaw mechanical sensitization, we proposed that this response in mCHI animals is centrally mediated.

Deposit of Biological Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, USA (ATCC):

Material	Antibody No.	ATCC Accession No.	Date of Deposit
pDb.CGRP.hFcGl	G1 heavy chain	PTA-6867	July 15, 2005
pEb.CGRP.hKGl	G1 light chain	PTA-6866	July 15, 2005

Vector pEb.CGRP.hKGl is a polynucleotide encoding the G1 light chain variable region and the light chain kappa constant region; and vector pDb.CGRP.hFcGl is a polynucleotide encoding the G1 heavy chain variable region and the heavy chain IgG2 constant region containing the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence; see Eur. J. Immunol. (1999) 29:2613-2624).

These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Rinat Neuroscience Corp. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to

the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC Section 122 and the Commissioner's rules pursuant thereto (including 37 CFR Section 1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Antibody Sequences

G1 heavy chain variable region amino acid sequence (SEQ ID NO:1)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWISWVRQAPGKGLEWVAEIRSESDA SATHYAEAVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCLAYFDYGLAIQNYWGQG TLVTVSS

G1 light chain variable region amino acid sequence (SEQ ID NO:2)

EIVLTQSPATLSLSPGERATLSCKASKRVTTYVSWYQQKPGQAPRLLIYGASNRYLGIP ARFSGSGSGTDFTLTISSLEPEDFAVYYCSQSYNYPYTFGQGTKLEIK

G1 CDR H1 (extended CDR) (SEQ ID NO:3)

GFTFSNYWIS

G1 CDR H2 (extended CDR) (SEQ ID NO:4)

EIRSESDASATHYAEAVKG

G1 CDR H3 (SEQ ID NO:5)

YFDYGLAIQNY

G1 CDR L1 (SEQ ID NO:6)
KASKRVTTYVS

G1 CDR L2 (SEQ ID NO:7)
GASNRYL

G1 CDR L3 (SEQ ID NO:8)
SQSYNYPYT

G1 heavy chain variable region nucleotide sequence (SEQ ID NO:9)
GAAGTTCAGCTGGTTGAATCCGGTGGTGGTCTGGTTCAGCCAGGTGGTTCCCTGC
GTCTGTCCTGCGCTGCTTCCGGTTTCACCTTCTCCAACTACTGGATCTCCTGGGTT
CGTCAGGCTCCTGGTAAAGGTCTGGAATGGGTTGCTGAAATCCGTTCCGAATCCG
ACGCGTCCGCTACCCATTACGCTGAAGCTGTTAAAGGTCGTTTCACCATCTCCCGT
GACAACGCTAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGTGCTGAAGACAC
CGCTGTTTACTACTGCCTGGCTTACTTTGACTACGGTCTGGCTATCCAGAACTACT
GGGGTCAGGGTACCCTGGTTACCGTTTCCTCC

G1 light chain variable region nucleotide sequence (SEQ ID NO:10)
GAAATCGTTCTGACCCAGTCCCCGGCTACCCTGTCCCTGTCCCCAGGTGAACGTGCT
ACCCTGTCCTGCAAAGCTTCCAAACGGGTTACCACCTACGTTTCCTGGTACCAGCAGA
AACCCGGTCAGGCTCCTCGTCTGCTGATCTACGGTGCTTCCAACCGTTACCTCGGTAT
CCCAGCTCGTTTCTCCGGTTCCGGTTCCGGTACCGACTTCACCCTGACCATCTCCTCC
CTGGAACCCGAAGACTTCGCTGTTTACTACTGCAGTCAGTCCTACAACTACCCCTACA
CCTTCGGTCAGGGTACCAAACTGGAAATCAAA

G1 heavy chain full antibody amino acid sequence (including modified IgG2 as described herein) (SEQ ID NO:11)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWISWVRQAPGKGLEWVAEIRSESDA
SATHYAEAVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCLAYFDYGLAIQNYWGQG
TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPC
PAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREP
QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGS
FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

G1 light chain full antibody amino acid sequence (SEQ ID NO:12)

EIVLTQSPATLSLSPGERATLSCKASKRVTTYVSWYQQKPGQAPRLLIYGASNRYLGIP
ARFSGSGSGTDFTLTISSLEPEDFAVYYCSQSYNYPYTFGQGTKLEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

G1 heavy chain full antibody nucleotide sequence (including modified IgG2 as described herein) (SEQ ID NO:13)

GAAGTTCAGCTGGTTGAATCCGGTGGTGGTCTGGTTCAGCCAGGTGGTTCCCTGC
GTCTGTCCTGCGCTGCTTCCGGTTTCACCTTCTCCAACTACTGGATCTCCTGGGTT
CGTCAGGCTCCTGGTAAAGGTCTGGAATGGGTTGCTGAAATCCGTTCCGAATCCG
ACGCGTCCGCTACCCATTACGCTGAAGCTGTTAAAGGTCGTTTCACCATCTCCCGT
GACAACGCTAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGTGCTGAAGACA
CCGCTGTTTACTACTGCCTGGCTTACTTTGACTACGGTCTGGCTATCCAGAACTAC
TGGGGTCAGGGTACCCTGGTTACCGTTTCCTCCGCCTCCACCAAGGGCCCATCTG
TCTTCCCACTGGCCCCATGCTCCCGCAGCACCTCCGAGAGCACAGCCGCCCTGG
GCTGCCTGGTCAAGGACTACTTCCCAGAACCTGTGACCGTGTCCTGGAACTCTGG
CGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTGCAGTCCTCAGGTCTC
TACTCCCTCAGCAGCGTGGTGACCGTGCCATCCAGCAACTTCGGCACCCAGACCT

ACACCTGCAACGTAGATCACAAGCCAAGCAACACCAAGGTCGACAAGACCGTGGA
GAGAAAGTGTTGTGTGGAGTGTCCACCTTGTCCAGCCCCTCCAGTGGCCGGACCA
TCCGTGTTCCTGTTCCCTCCAAAGCCAAAGGACACCCTGATGATCTCCAGAACCCC
AGAGGTGACCTGTGTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGTGCAGTT
CAACTGGTATGTGGACGGAGTGGAGGTGCACAACGCCAAGACCAAGCCAAGAGA
GGAGCAGTTCAACTCCACCTTCAGAGTGGTGAGCGTGCTGACCGTGGTGCACCAG
GACTGGCTGAACGGAAAGGAGTATAAGTGTAAGGTGTCCAACAAGGGACTGCCAT
CCAGCATCGAGAAGACCATCTCCAAGACCAAGGGACAGCCAAGAGAGCCACAGGT
GTATACCCTGCCCCCATCCAGAGAGGAGATGACCAAGAACCAGGTGTCCCTGACC
TGTCTGGTGAAGGGATTCTATCCATCCGACATCGCCGTGGAGTGGGAGTCCAACG
GACAGCCAGAGAACAACTATAAGACCACCCCTCCAATGCTGGACTCCGACGGATC
CTTCTTCCTGTATTCCAAGCTGACCGTGGACAAGTCCAGATGGCAGCAGGGAAAC
GTGTTCTCTTGTTCCGTGATGCACGAGGCCCTGCACAACCACTATACCCAGAAGAG
CCTGTCCCTGTCTCCAGGAAAGTAA

G1 light chain full antibody nucleotide sequence (SEQ ID NO:14)

GAAATCGTTCTGACCCAGTCCCCGGCTACCCTGTCCCTGTCCCCAGGTGAACGTG
CTACCCTGTCCTGCAAAGCTTCCAAACGGGTTACCACCTACGTTTCCTGGTACCAG
CAGAAACCCGGTCAGGCTCCTCGTCTGCTGATCTACGGTGCTTCCAACCGTTACCT
CGGTATCCCAGCTCGTTTCTCCGGTTCCGGTTCCGGTACCGACTTCACCCTGACC
ATCTCCTCCCTGGAACCCGAAGACTTCGCTGTTTACTACTGCAGTCAGTCCTACAA
CTACCCCTACACCTTCGGTCAGGGTACCAAACTGGAAATCAAACGCACTGTGGCT
GCACCATCTGTCTTCATCTTCCCTCCATCTGATGAGCAGTTGAAATCCGGAACTGC
CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCGCGCGAGGCCAAAGTACAGTGGA
AGGTGGATAACGCCCTCCAATCCGGTAACTCCCAGGAGAGTGTCACAGAGCAGGA
CAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACCCTGAGCAAAGCAGAC
TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGTTCTC
CAGTCACAAAGAGCTTCAACCGCGGTGAGTGCTAA

Amino acid sequence comparison of human and rat CGRP (human α-CGRP (SEQ ID NO:15); human β-CGRP (SEQ ID NO:43); rat α-CGRP (SEQ ID NO:41); and rat β-CGRP (SEQ ID NO:44)):

NH_2-ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-$CONH_2$ (human α-CGRP)

NH_2-ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF-$CONH_2$ (human β-CGRP)

NH_2-SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSEAF-$CONH_2$ (rat α-CGRP)

NH_2-SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSKAF-$CONH_2$ (rat β-CGRP)

Light chain variable region LCVR17 amino acid sequence (SEQ ID NO:58)

DIQMTQSPSSLSASVGDRVTITCRASQDIDNYLNWYQQKPGKAPKLLIYYTSEYHSGV PSRFSGSGSGTDFTFTISSLQPEDIATYYCQQGDALPPTFGQGTKLEIK

Heavy chain variable region HCVR22 amino acid sequence (SEQ ID NO:59)

QVQLVQSGAEVKKPGASVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT GDTRYIQKFAGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARLSDYVSGFSYWGQG TLVTVSS

Light chain variable region LCVR18 amino acid sequence (SEQ ID NO:60)

DIQMTQSPSSLSASVGDRVTITCRASQDIDNYLNWYQQKPGKAPKLLIYYTSEYHSGV PSRFSGSGSGTDFTFTISSLQPEDIATYYCQQGDALPPTFGQGTKLEIK

Heavy chain variable region HCVR23 amino acid sequence (SEQ ID NO:61)

QVQLVQSGAEVKKPGASVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT GKTVYIQKFAGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARLSDYVSGFSYWGQG TLVTVSS

Light chain variable region LCVR19 amino acid sequence (SEQ ID NO:62)

DIQMTQSPSSLSASVGDRVTITCRASKDISKYLNWYQQKPGKAPKLLIYYTSGYHSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDALPPTFGGGTKVEIK

Heavy chain variable region HCVR24 amino acid sequence (SEQ ID NO:63)
QVQLVQSGAEVKKPGSSVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT
GKTVYIQKFADRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQGT
TVTVSS

Light chain variable region LCVR20 amino acid sequence (SEQ ID NO:64)
DIQMTQSPSSLSASVGDRVTITCRASRPIDKYLNWYQQKPGKAPKLLIYYTSEYHSGVP
SRFSGSGSGTDFTFTISSLQPEDIATYYCQQGDALPPTFGQGTKLEIK

Heavy chain variable region HCVR25 amino acid sequence (SEQ ID NO:65)
QVQLVQSGAEVKKPGASVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT
GKTVYIQKFAGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQG
TLVTVSS

Light chain variable region LCVR21 amino acid sequence (SEQ ID NO:66)
DIQMTQSPSSLSASVGDRVTITCRASQDIDKYLNWYQQKPGKAPKLLIYYTSGYHSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDALPPTFGGGTKVEIK

Heavy chain variable region HCVR26 amino acid sequence (SEQ ID NO:67)
QVQLVQSGAEVKKPGSSVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT
GKTVYIQKFAGRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQGT
TVTVSS

Light chain variable region LCVR27 amino acid sequence (SEQ ID NO:68)
QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASG
VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIKR

Heavy chain variable region HCVR28 amino acid sequence (SEQ ID NO:69)
EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGAT
YYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS

Light chain variable region LCVR29 amino acid sequence (SEQ ID NO:70)
QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTLASG
VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSSGDCFVFGGGTKVEIKR

Heavy chain variable region HCVR30 amino acid sequence (SEQ ID NO:71)
EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGINDN
TYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS

Light chain variable region LCVR31 amino acid sequence (SEQ ID NO:72)
QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTLASG
VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSSGDCFVFGGGTKVEIKR

Heavy chain variable region HCVR32 amino acid sequence (SEQ ID NO:73)
EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGINDN
TYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS

Light chain variable region LCVR33 amino acid sequence (SEQ ID NO:74)
QVLTQTPSPVSAAVGSTVTINCQASQSVYHNTYLAWYQQKPGQPPKQLIYDASTLASG
VPSRFSGSGSGTQFTLTISGVQCNDAAAYYCLGSYDCTNGDCFVFGGGTEVVVKR

Heavy chain variable region HCVR34 amino acid sequence (SEQ ID NO:75)
QSLEESGGRLVTPGTPLTLTCSVSGIDLSGYYMNWVRQAPGKGLEWIGVIGINGATYY
ASWAKGRFTISKTSSTTVDLKMTSLTTEDTATYFCARGDIWGPGTLVTVSS

Light chain variable region LCVR35 amino acid sequence (SEQ ID NO:76)
QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASG
VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIKR

Heavy chain variable region HCVR36 amino acid sequence (SEQ ID NO:77)

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGAT
YYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS

Light chain variable region LCVR37 amino acid sequence (SEQ ID NO:78)

QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSG
IPDRFSGSKSGTSTTLGITGLQTGDEADYYCGTWDSRLSAVVFGGGTKLTVL

Heavy chain variable region HCVR38 amino acid sequence (SEQ ID NO:79)

QVQLVESGGGVVQPGRSLRLSCAASGFTFSSFGMHWVRQAPGKGLEWVAVISFDGS
IKYSVDSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCARDRLNYYDSSGYYHYKY
YGMAVWGQGTTVTVSS

WHAT IS CLAIMED IS:

1. A composition comprising a monoclonal antibody that modulates the CGRP pathway for use in treating or reducing incidence of post-traumatic headache in a subject.

2. A composition comprising a monoclonal antibody that modulates the CGRP pathway for use in treating or reducing incidence of at least one secondary symptom associated with post-traumatic headache in a subject.

3. The composition of claim 1 or 2, wherein the composition comprises about 225 mg to about 1000 mg of the monoclonal antibody.

4. The composition of any one of the previous claims, wherein the composition is administered monthly.

5. The composition of claim 4, wherein the composition is administered monthly for three months or more.

6. The composition of claim 4 or 5, wherein the dose of the monoclonal antibody administered to the subject is about 225 mg to about 675 mg.

7. The composition of any one of claims 1-4, wherein the composition is administered as a single dose.

8. The composition of claim 7, wherein the dose of the monoclonal antibody administered to the subject is about 675 mg to about 1000 mg.

9. The composition of any one of the previous claims, wherein the treating or reducing comprises reduction in the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing a 6-item Headache Impact Test (HIT-6) disability score, or any combination thereof.

10. The composition of claim 9, wherein the number of monthly headache days is reduced by at least about 50% from a pre-administration level in the subject.

11. The composition of any one of the previous claims, wherein the monoclonal antibody is an anti-CGRP antagonist antibody.

12. The composition of any one of the previous claims, wherein the composition is administered subcutaneously or intravenously.

13. The composition of any one of the previous claims, wherein the composition is administered utilizing a pre-filled syringe comprising a dose of the monoclonal antibody.

14. The composition of any one of the previous claims, wherein the monoclonal antibody is formulated at a concentration of at least 150 mg/mL.

15. The composition of any one of the previous claims, wherein the monoclonal antibody is administered in a volume of less than 2 mL.

16. The composition of any one of the previous claims, wherein the composition is administered to the subject with a second agent simultaneously or sequentially with the monoclonal antibody.

17. The composition of claim 16, wherein monthly use of the second agent by the subject is decreased by at least 15% after administering the monoclonal antibody.

18. The composition of any one of the previous claims, wherein the subject is human.

19. The composition of any one of the previous claims, wherein the monoclonal antibody is human or humanized.

20. The composition of any one of the previous claims, wherein the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

21. A composition comprising a therapeutically effective amount of a monoclonal antibody that modulates the calcitonin gene-related peptide (CGRP) pathway for use in preventing, treating, or reducing incidence of a post-traumatic headache in a subject who has suffered a mild traumatic brain injury.

22. The composition of claim 21, wherein the monoclonal antibody is administered to the subject intravenously or subcutaneously.

23. The composition of claim 21, wherein the monoclonal antibody is administered to the subject intravenously in an infusion over about one hour.

24. The composition of claim 21, wherein one or more additional doses of the composition is administered to the subject.

25. The composition of claim 21, wherein the monoclonal antibody is administered at a dose of about 675 mg.

26. The composition of claim 25, wherein the monoclonal antibody is administered to the subject at a dose of about 225 mg on the following two months.

27. The composition of claim 21, wherein the monoclonal antibody is administered at a dose of about 900 mg.

28. The composition of claim 27, wherein the monoclonal antibody is administered at a dose of about 900 mg quarterly.

29. The composition of claim 21, wherein the monoclonal antibody is administered immediately after the brain injury.

30. The composition of claim 21, wherein the treating or reducing comprises reducing the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing neck pain, reducing a 6-item Headache Impact Test (HIT-6) disability score, improving 12-Item Short Form Health Survey (SF-12) score, improving on Patient Global Impression of Change (PGIC) scale, improving Sport ConCuSSion ASSeSment tool 3 (SCAT-3) score, or any combination thereof.

31. The composition of claim 21, wherein the antibody is administered to the subject from a pre-filled syringe, pre-filled syringe with a needle safety device, injection pen, or auto-injector comprising a dose of the monoclonal antibody.

32. The composition of claim 21, wherein the monoclonal antibody is administered as a formulation comprising the antibody at a concentration of at least about 150 mg/mL.

33. The composition of claim 21, wherein the monoclonal antibody is administered in a volume of less than 2 mL.

34. The composition of claim 21, wherein the composition is administered to the subject with a second agent simultaneously or sequentially with the monoclonal antibody.

35. The composition of claim 34, wherein the second agent is selected from the group consisting of a 5-HT1 agonist, triptan, opiate, β-adrenergic antagonist, ergot alkaloid, and non-steroidal anti-inflammatory drug (NSAID).

36. The composition of claim 35, wherein the triptan is sumatriptan, zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan, or frovatriptan.

37. The composition of claim 21, wherein the monoclonal antibody is an anti-CGRP antagonist antibody.

38. The composition of claim 21, wherein the monoclonal antibody is human or humanized.

39. The composition of claim 21, wherein the monoclonal antibody is a humanized anti-CGRP antagonist antibody.

40. The composition of claim 21, wherein the monoclonal antibody comprises a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8.

41. The composition of claim 21, wherein the monoclonal antibody is an IgG1, IgG2, IgG3, or IgG4 antibody.

42. The composition of claim 21, wherein the subject is human.

43. A method of treating or reducing incidence of post-traumatic headache in a subject comprising administering to the subject a monoclonal antibody that modulates the CGRP pathway.

44. A method of treating or reducing incidence of at least one secondary symptom associated with post-traumatic headache in a subject comprising administering to the subject a monoclonal antibody that modulates the CGRP pathway.

45. The method of claim 43 or 44, wherein the amount of the monoclonal antibody administered to the subject is about 225 mg to about 1000 mg.

46. The method of any one of the previous claims, wherein the monoclonal antibody is administered monthly.

47. The method of claim 46, wherein the monoclonal antibody is administered monthly for three months or more.

48. The method of claim 46 or 47, wherein the dose of the monoclonal antibody administered to the subject is about 225 mg to about 675 mg.

49. The method of any one of claims 43-46, wherein the monoclonal antibody is administered as a single dose.

50. The method of claim 49, wherein the dose of the monoclonal antibody administered to the subject is about 675 mg to about 1000 mg.

51. The method of any one of the previous claims, wherein the treating or reducing comprises reduction in the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing a 6-item Headache Impact Test (HIT-6) disability score, or any combination thereof.

52. The method of claim 51, wherein the number of monthly headache days is reduced by at least about 50% from a pre-administration level in the subject.

53. The method of any one of the previous claims, wherein the monoclonal antibody is an anti-CGRP antagonist antibody.

54. The method of any one of the previous claims, wherein the administering is subcutaneous or intravenous administration.

55. The method of any one of the previous claims, wherein the administering comprises utilizing a pre-filled syringe comprising a dose of the monoclonal antibody.

56. The method of any one of the previous claims, wherein the monoclonal antibody is formulated at a concentration of at least 150 mg/mL.

57. The method of any one of the previous claims, wherein the monoclonal antibody is administered in a volume of less than 2 mL.

58. The method of any one of the previous claims, comprising administering to the subject a second agent simultaneously or sequentially with the monoclonal antibody.

59. The method of claim 58, wherein monthly use of the second agent by the subject is decreased by at least 15% after administering the monoclonal antibody.

60. The method of any one of the previous claims, wherein the subject is human.

61. The method of any one of the previous claims, wherein the monoclonal antibody is human or humanized.

62. The method of any one of the previous claims, wherein the monoclonal antibody comprises (a) an antibody having a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8; or (b) a variant of an antibody according to (a) as shown in Table 6.

63. A method of preventing, treating, or reducing incidence of a post-traumatic headache in a subject, the method comprising:

selecting a subject who has suffered a mild traumatic brain injury; and

administering to the subject a therapeutically effective amount of a monoclonal antibody that modulates the calcitonin gene-related peptide (CGRP) pathway.

64. The method of claim 63, wherein the monoclonal antibody is administered to the subject intravenously or subcutaneously.

65. The method of claim 63, wherein the monoclonal antibody is administered to the subject intravenously in an infusion over about one hour.

66. The method of claim 63, further comprising administering one or more additional doses of the monoclonal antibody to the subject.

67. The method of claim 63, wherein the monoclonal antibody is administered at a dose of about 675 mg.

68. The method of claim 67, further comprising administering to the subject the monoclonal antibody at a dose of about 225 mg on the following two months.

69. The method of claim 63, wherein the monoclonal antibody is administered at a dose of about 900 mg.

70. The method of claim 69, wherein the monoclonal antibody is administered at a dose of about 900 mg quarterly.

71. The method of claim 63, wherein the monoclonal antibody is administered immediately after the brain injury.

72. The method of claim 63, wherein the treating or reducing comprises reducing the number of headache hours of any severity, reducing the number of monthly headache days of any severity, reducing the use of any acute headache medications, reducing neck pain, reducing a 6-item Headache Impact Test (HIT-6) disability score, improving 12-Item Short Form Health Survey (SF-12) score, improving on Patient Global Impression of Change (PGIC) scale, improving Sport ConCuSSion ASSeSment tool 3 (SCAT-3) score, or any combination thereof.

73. The method of claim 63, wherein the administering comprises administering the antibody to the subject from a pre-filled syringe, pre-filled syringe with a needle safety device, injection pen, or auto-injector comprising a dose of the monoclonal antibody.

74. The method of claim 63, wherein the monoclonal antibody is administered as a formulation comprising the antibody at a concentration of at least about 150 mg/mL.

75. The method of claim 63, wherein the monoclonal antibody is administered in a volume of less than 2 mL.

76. The method of claim 63, further comprising administering to the subject a second agent simultaneously or sequentially with the monoclonal antibody.

77. The method of claim 76, wherein the second agent is selected from the group consisting of a 5-HT1 agonist, triptan, opiate, β-adrenergic antagonist, ergot alkaloid, and non-steroidal anti-inflammatory drug (NSAID).

78. The method of claim 77, wherein the triptan is sumatriptan, zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan, or frovatriptan.

79. The method of claim 63, wherein the monoclonal antibody is an anti-CGRP antagonist antibody.

80. The method of claim 63, wherein the monoclonal antibody is human or humanized.

81. The method of claim 63, wherein the monoclonal antibody is a humanized anti-CGRP antagonist antibody.

82. The method of claim 63, wherein the monoclonal antibody comprises a CDR H1 as set forth in SEQ ID NO:3; a CDR H2 as set forth in SEQ ID NO:4; a CDR H3 as set forth in SEQ ID NO:5; a CDR L1 as set forth in SEQ ID NO:6; a CDR L2 as set forth in SEQ ID NO:7; and a CDR L3 as set forth in SEQ ID NO:8.

83. The method of claim 63, wherein the monoclonal antibody is an IgG1, IgG2, IgG3, or IgG4 antibody.

84. The method of claim 63, wherein the subject is human.

Figure 1

Fab	K_D (nM)	K_D (nM)	K_D (nM)	K_D (mutant/parent)										
	1-37 (WT)	19-37[a]	25-37[a]	F27A	V28A	P29A	T30A	N31A	V32A	G33A	S34A	K35A	F37A
7E9	1.0	1.1±0.8	*0.14±0.05*	1.0	1.0	26	7	9	41	1256	69	4	1598
8B6	1.1	1.5±1.2	*0.45±0.08*	1.0	1.0	9	22	3	5	496	26	3	2537
10A8	2.1	2.4±1.4	*1.0±0.2*	1.0	1.0	9	4	4	11	36	82	13	2162
7D11	4.4	10±7	*3.4±0.4*	1.1	1.0	7	4	5	5	86	18	1.4	420
6H2	9.3	7.8±0.2	*8.5±0.5*	0.9	1.0	1.0	0.8	4	11	14	0.5	1.0	
4901	60.5	52±12	*296±115*	0.8	0.8	0.2	0.2	0.3	0.9	1.3	0.8	0.3	
14E10	79.7	91±3	*117.4±0.7*	0.8	0.8	11	3	18	2	1	3	0.4[b]	
9B8	84.7	76±20	*96±28*	0.8	0.8	0.6	0.6	0.7	0.6	1.3	4	0.4[b]	
13C2	94.4	86±13	*137±5*	0.7	0.7	0.5	0.4	0.6	0.2	0.9	1.1	0.4[b]	
14A9	148.4	219±114	*246±20*	0.8	0.7	0.7	0.5	0.8	0.7	1.6	1.3	6	
6D5	209.9	207±26	*378±22*	0.8	0.7	0.5	0.4	0.6	0.5	3	1.1	5	
1C5	296.4	223±51	*430±173*	0.8	0.8	0.6	0.4	0.6	0.6	1.1	1.1	5	

Figure 2A

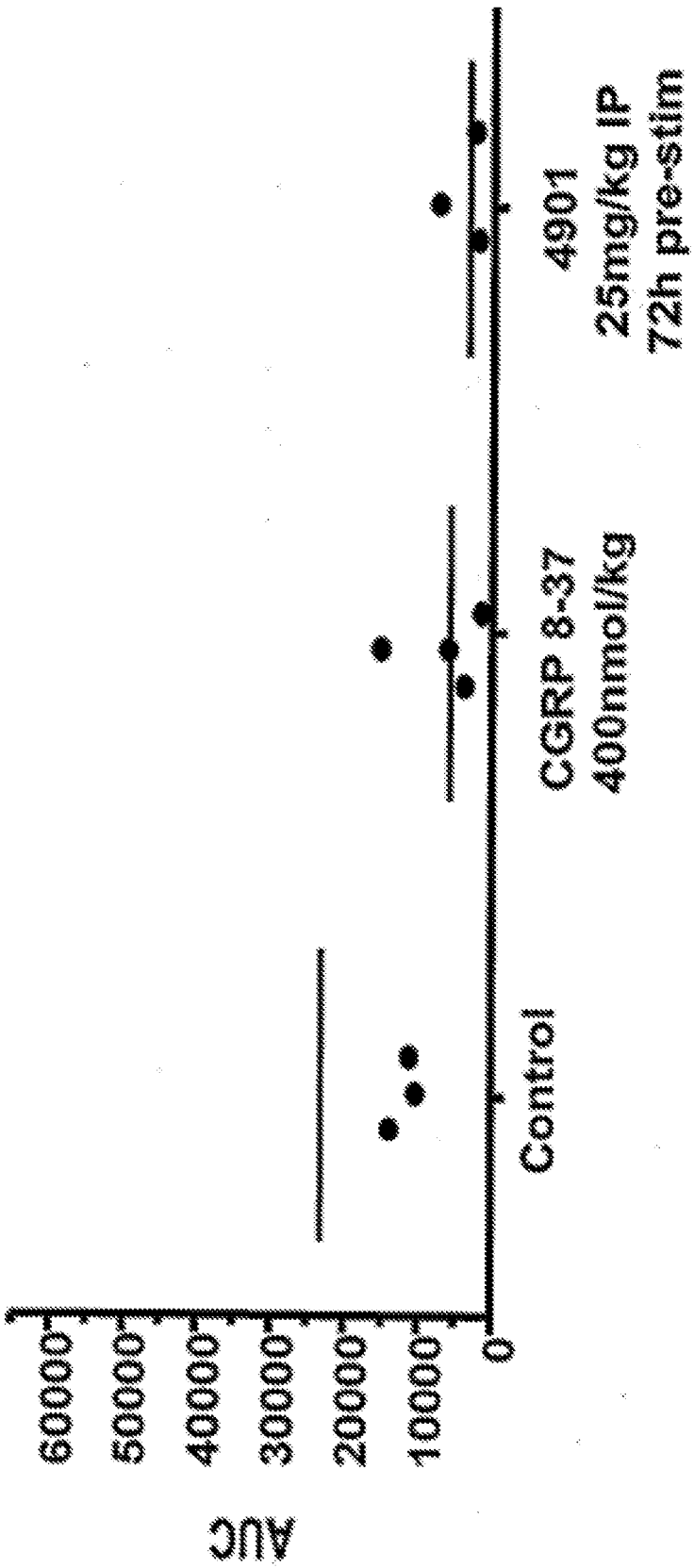

AUC
60000
50000
40000
30000
20000
10000
0
Control
CGRP 8-37
400nmol/kg
4901
25mg/kg IP
72h pre-stim

Figure 2B

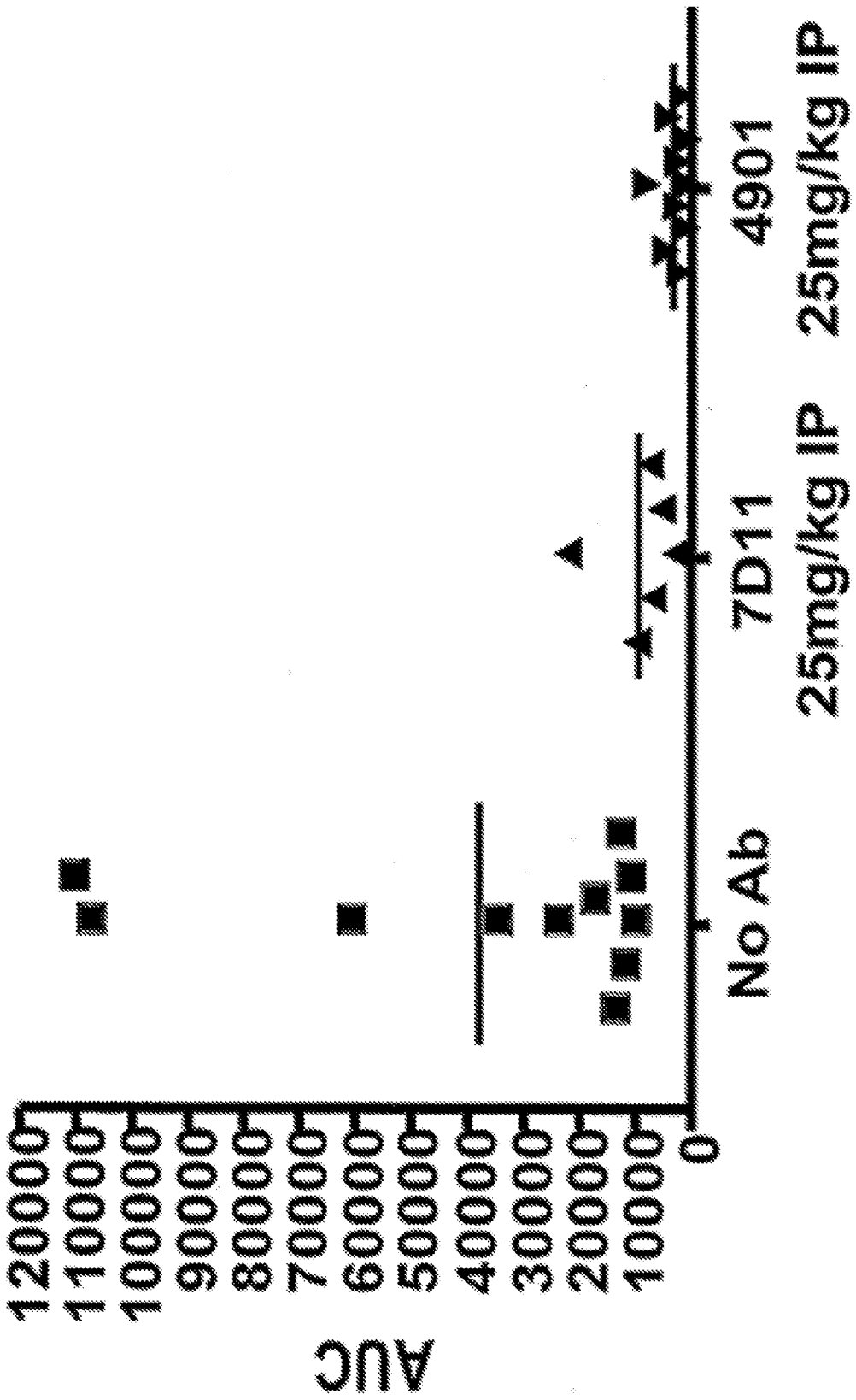

AUC
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
No Ab
7D11
4901
25mg/kg IP
25mg/kg IP

Figure 3

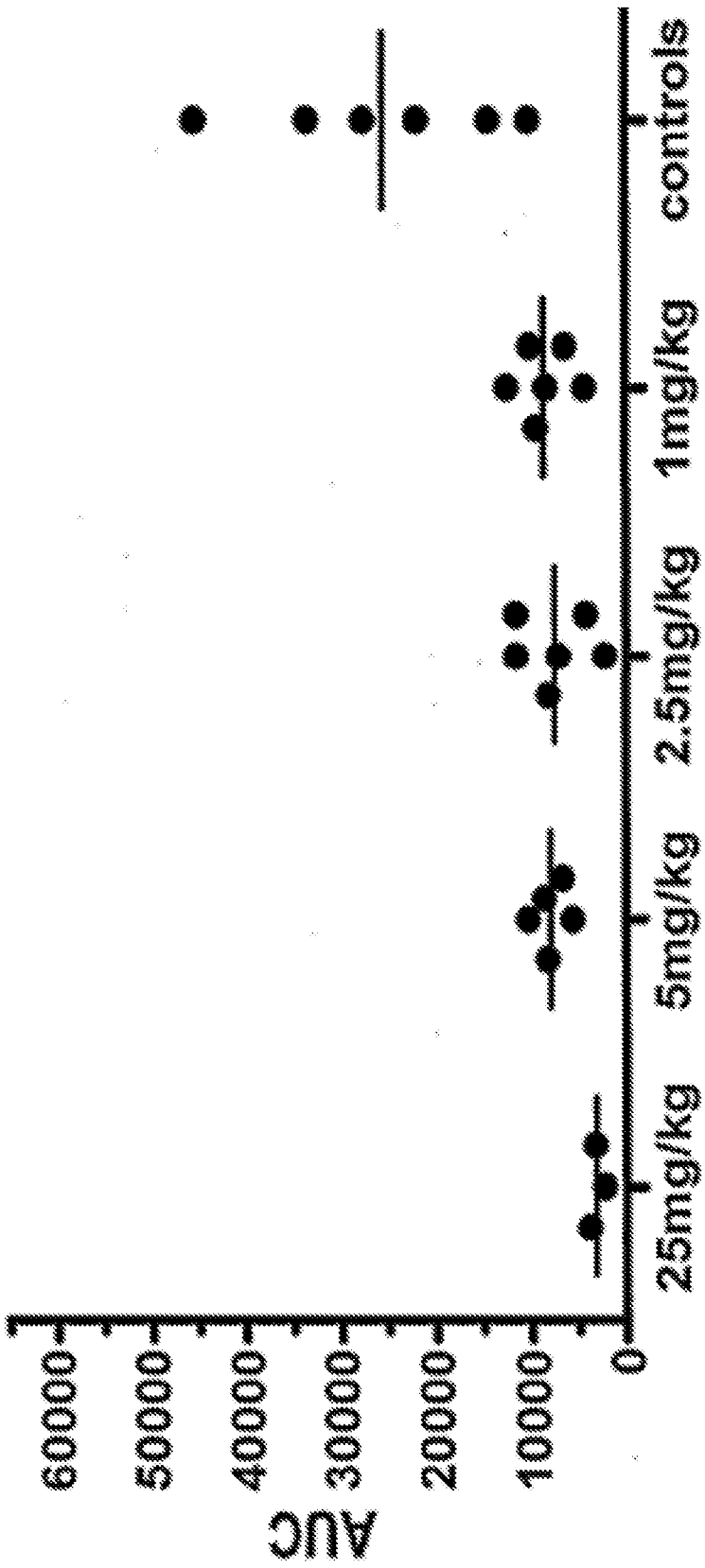
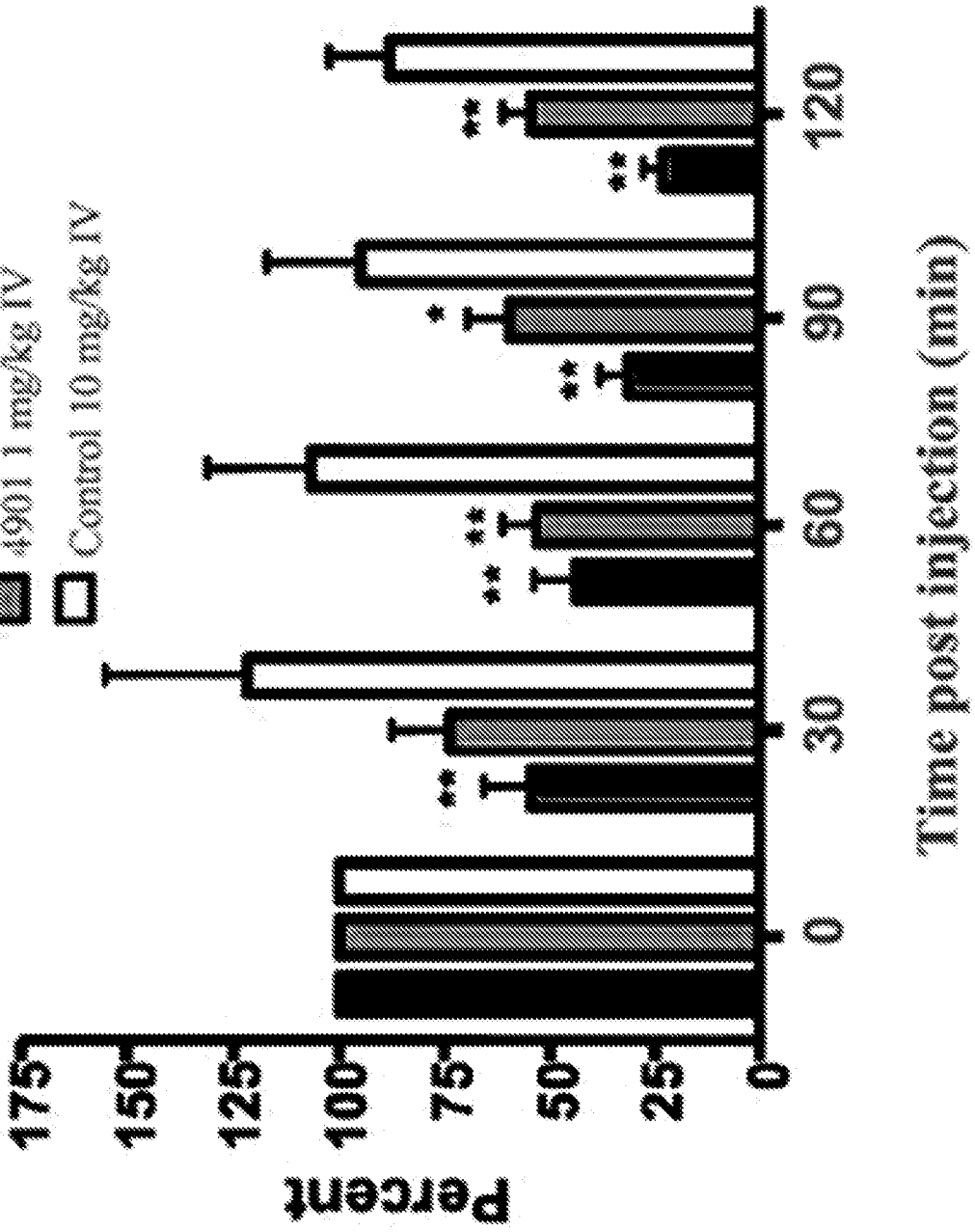

AUC
60000
50000
40000
30000
20000
10000
0
25mg/kg
5mg/kg
2.5mg/kg
1mg/kg
controls

Figure 4A


4901 10 mg/kg IV
4901 1 mg/kg IV
Control 10 mg/kg IV
Percent
175
150
125
100
75
50
25
0
0
30
60
90
120
Time post injection (min)
**
**
**
*
**
**

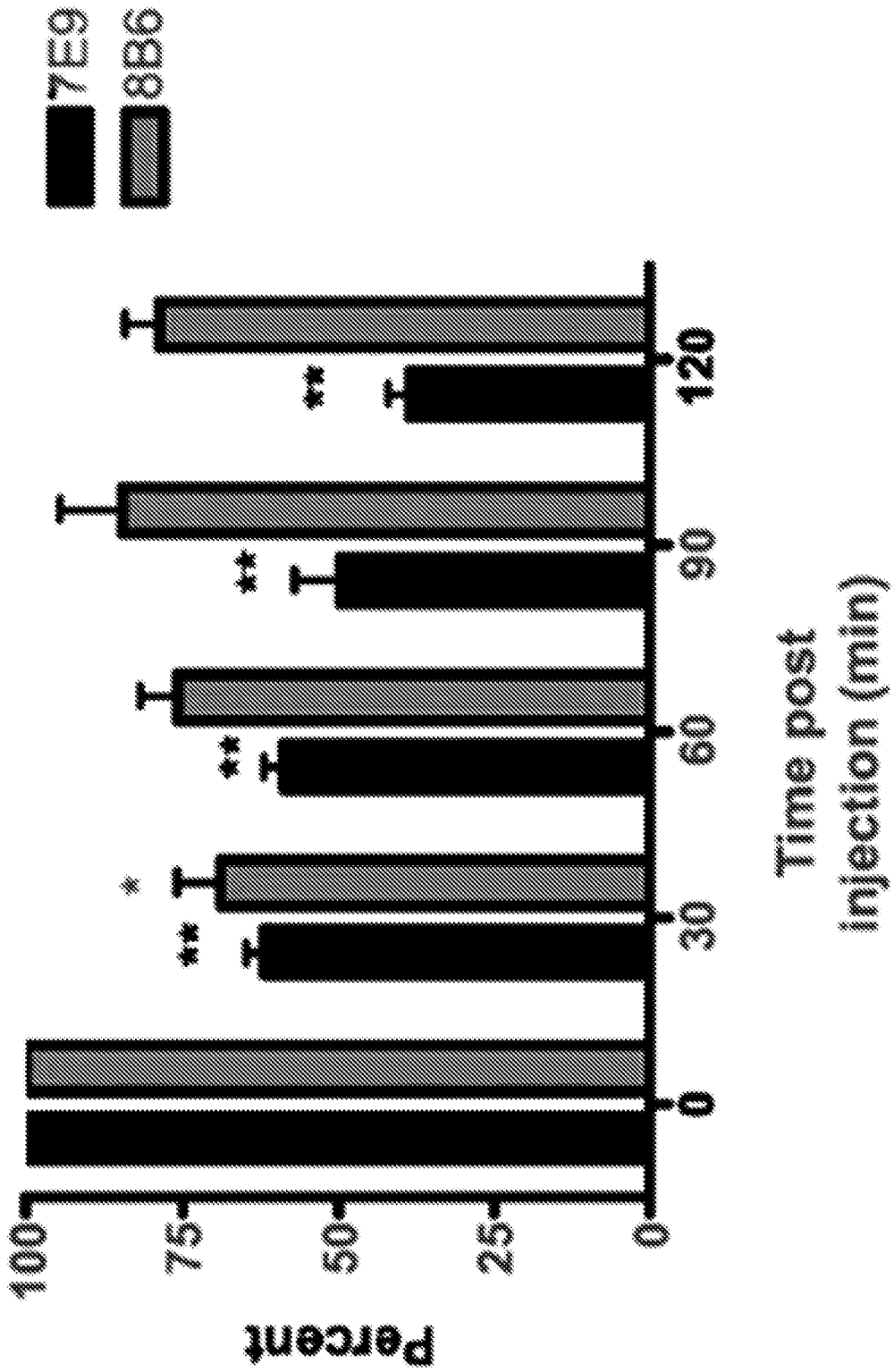
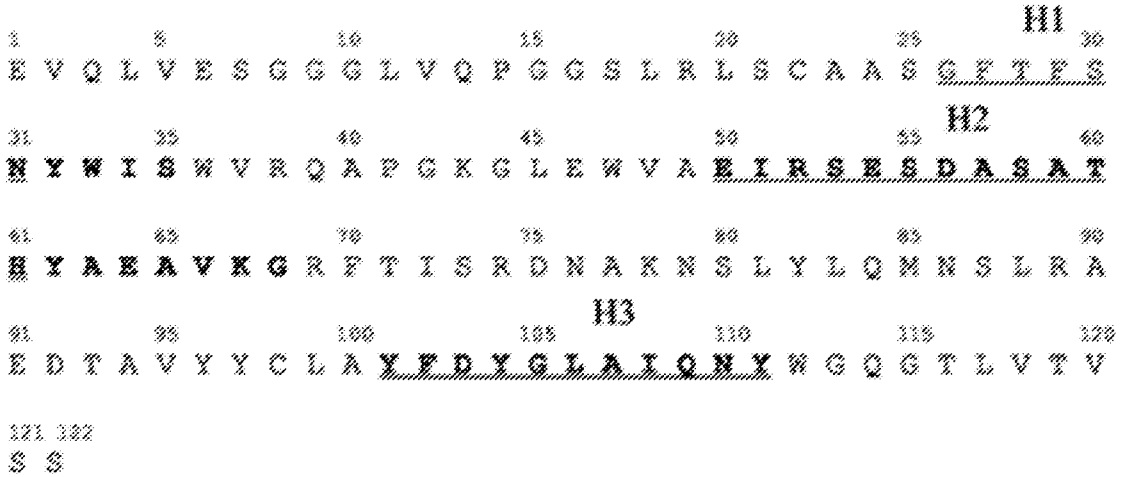

7E9
8B6
Percent
100
75
50
25
0
0
30
60
90
120
Time post injection (min)
*
**
**
**
**
**

Figure 4B

Figure 5

Bold=Kabat CDR
Underline=Chothia CDR

G1 Heavy chain



G1 Light chain

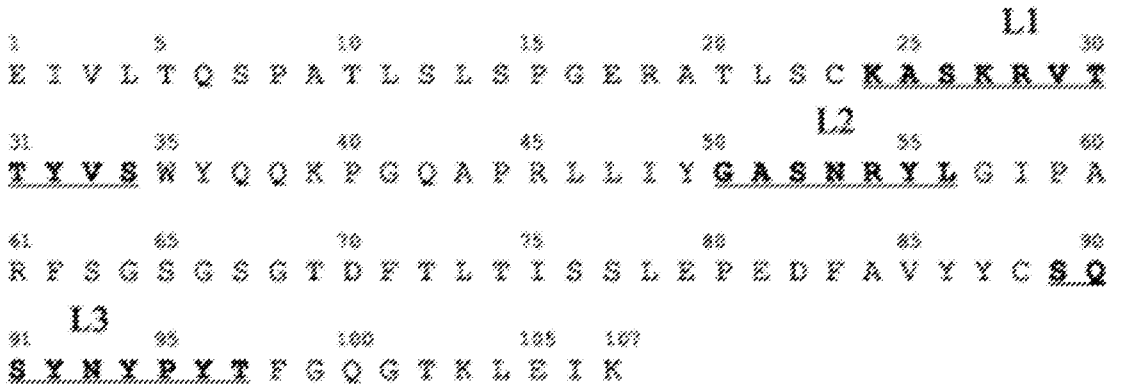


Figure 6

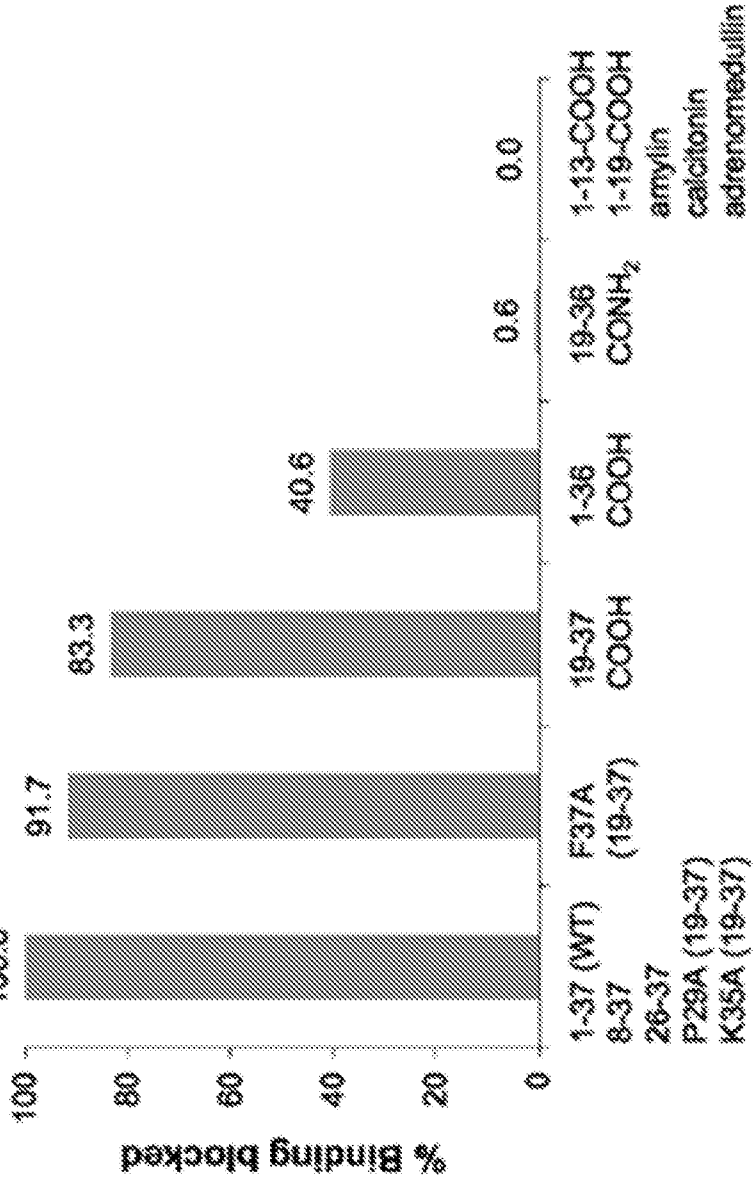

% Binding blocked
100
80
60
40
20
0
100.0
91.7
83.3
40.6
0.6
0.0
1-37 (WT)
8-37
26-37
P29A (19-37)
K35A (19-37)
K35E (19-37)
K35M (19-37)
1-37 β-human (WT)
1-37 α-rat (WT)
1-37 β-rat (WT)
F37A (19-37)
19-37 COOH
1-36 COOH
19-36 CONH2
1-13-COOH
1-19-COOH
amylin
calcitonin
adrenomedullin
Competing Peptides

Figure 7

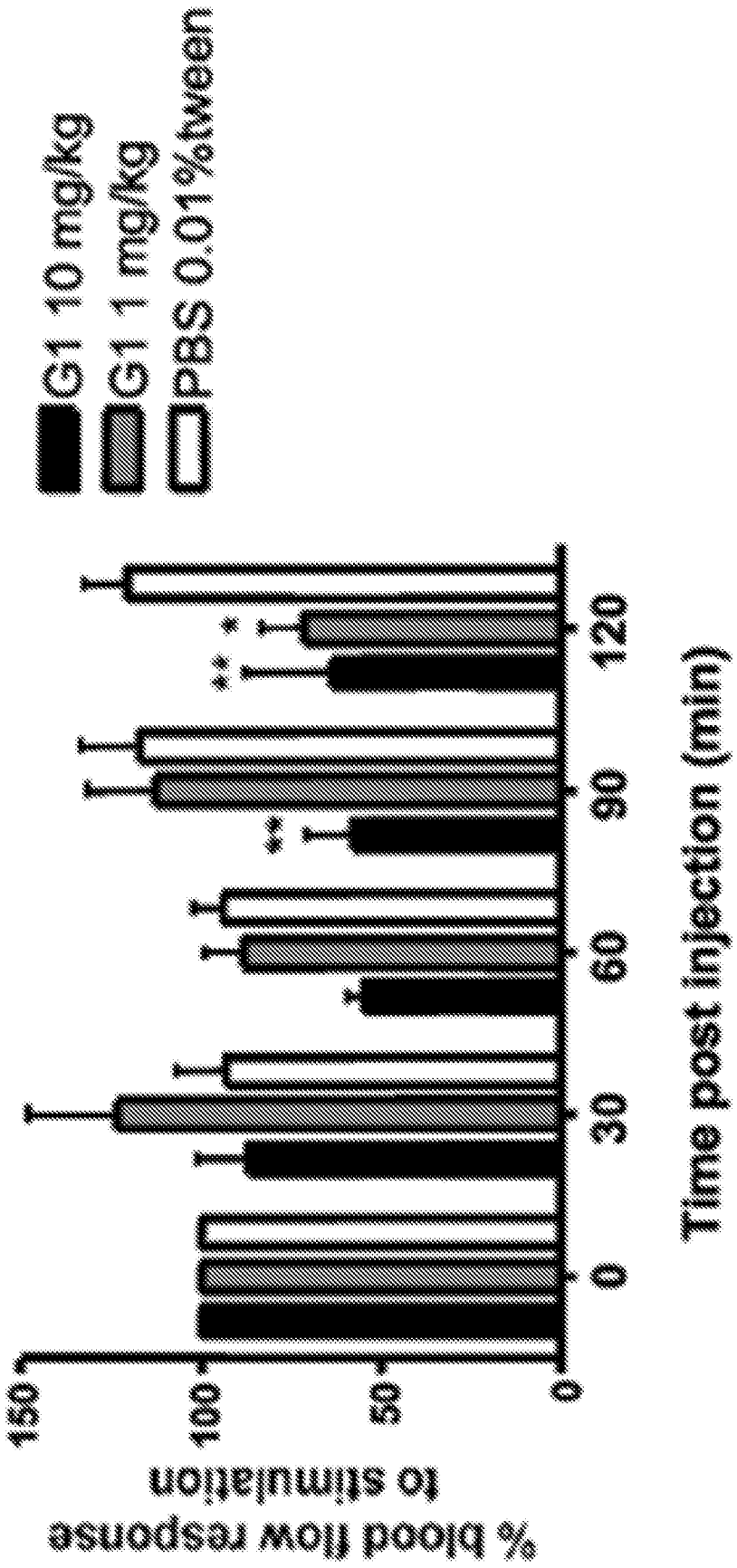

G1 10 mg/kg
G1 1 mg/kg
PBS 0.01%tween
% blood flow response to stimulation
150
100
50
0
0
30
60
90
120
Time post injection (min)
*
**
**

Figure 8A

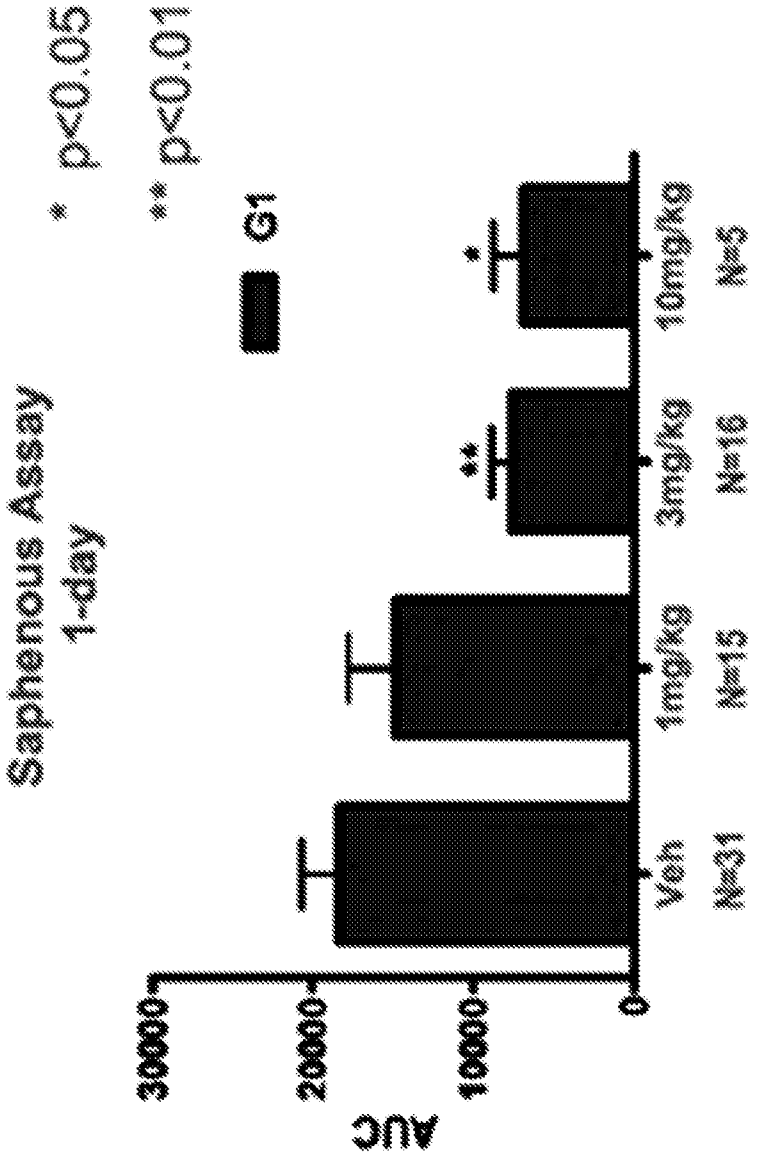

Saphenous Assay
1-day
* p<0.05
** p<0.01
G1
AUC
30000
20000
10000
0
Veh
N=31
1mg/kg
N=15
3mg/kg
N=16
10mg/kg
N=5
*
**
*

Figure 8B

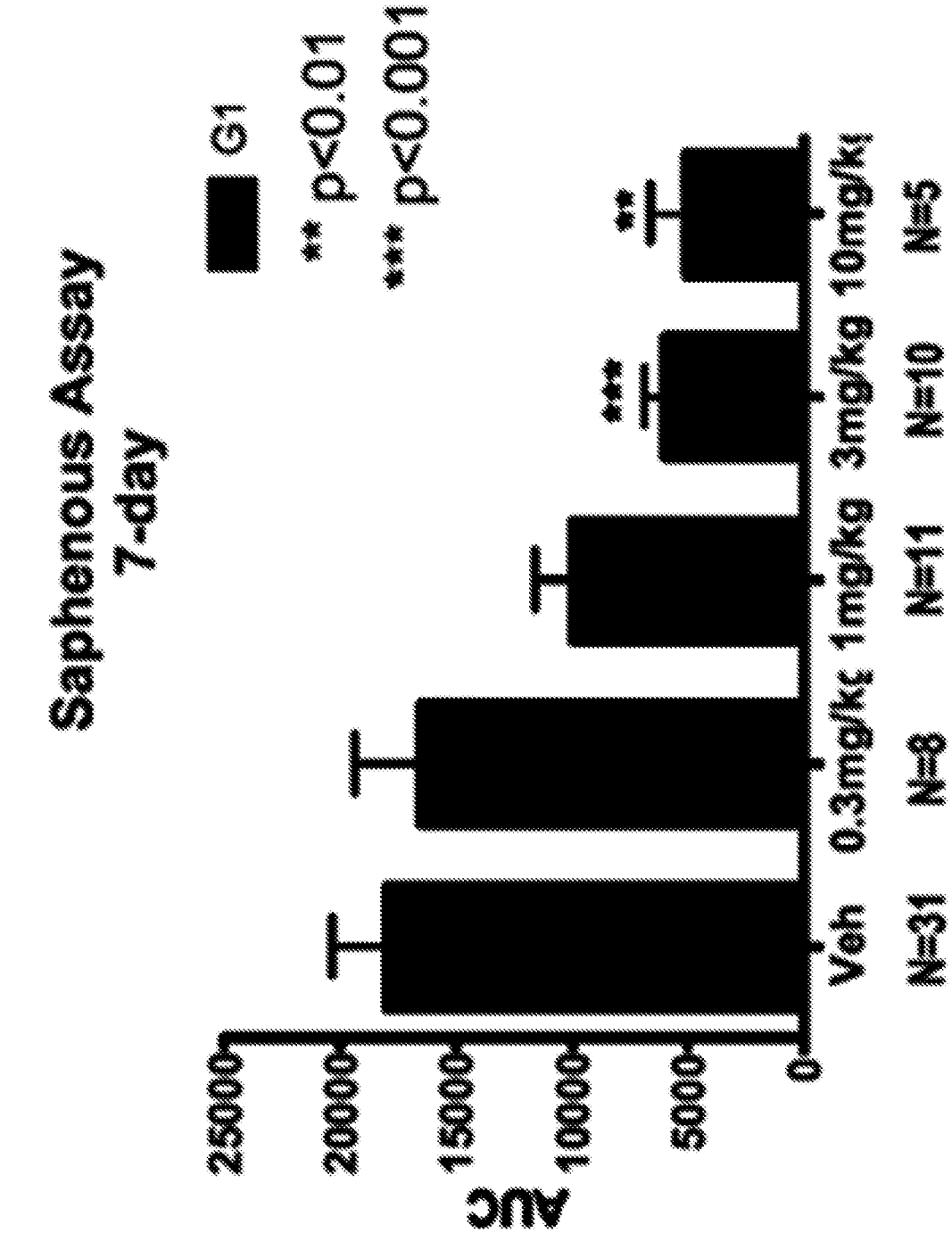

Saphenous Assay
7-day
G1
** p<0.01
*** p<0.001
AUC
25000
20000
15000
10000
5000
0
Veh
0.3mg/kg
1mg/kg
3mg/kg
10mg/kg
N=31
N=8
N=11
N=10
N=5
**
***
**

Figure 8C

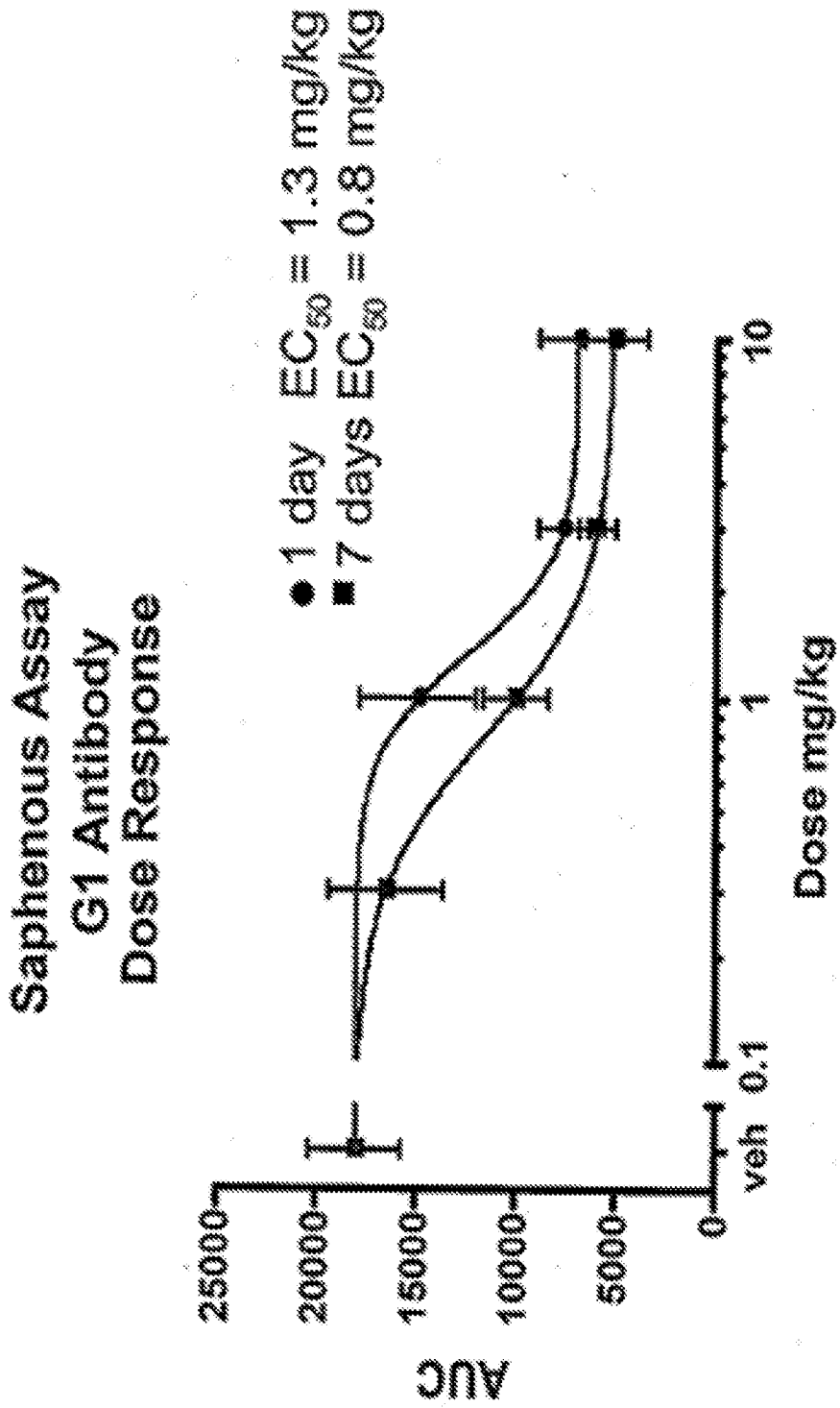

Saphenous Assay
G1 Antibody
Dose Response
● 1 day EC50 = 1.3 mg/kg
■ 7 days EC50 = 0.8 mg/kg
AUC
25000
20000
15000
10000
5000
0
veh
0.1
1
10
Dose mg/kg

Figure 9

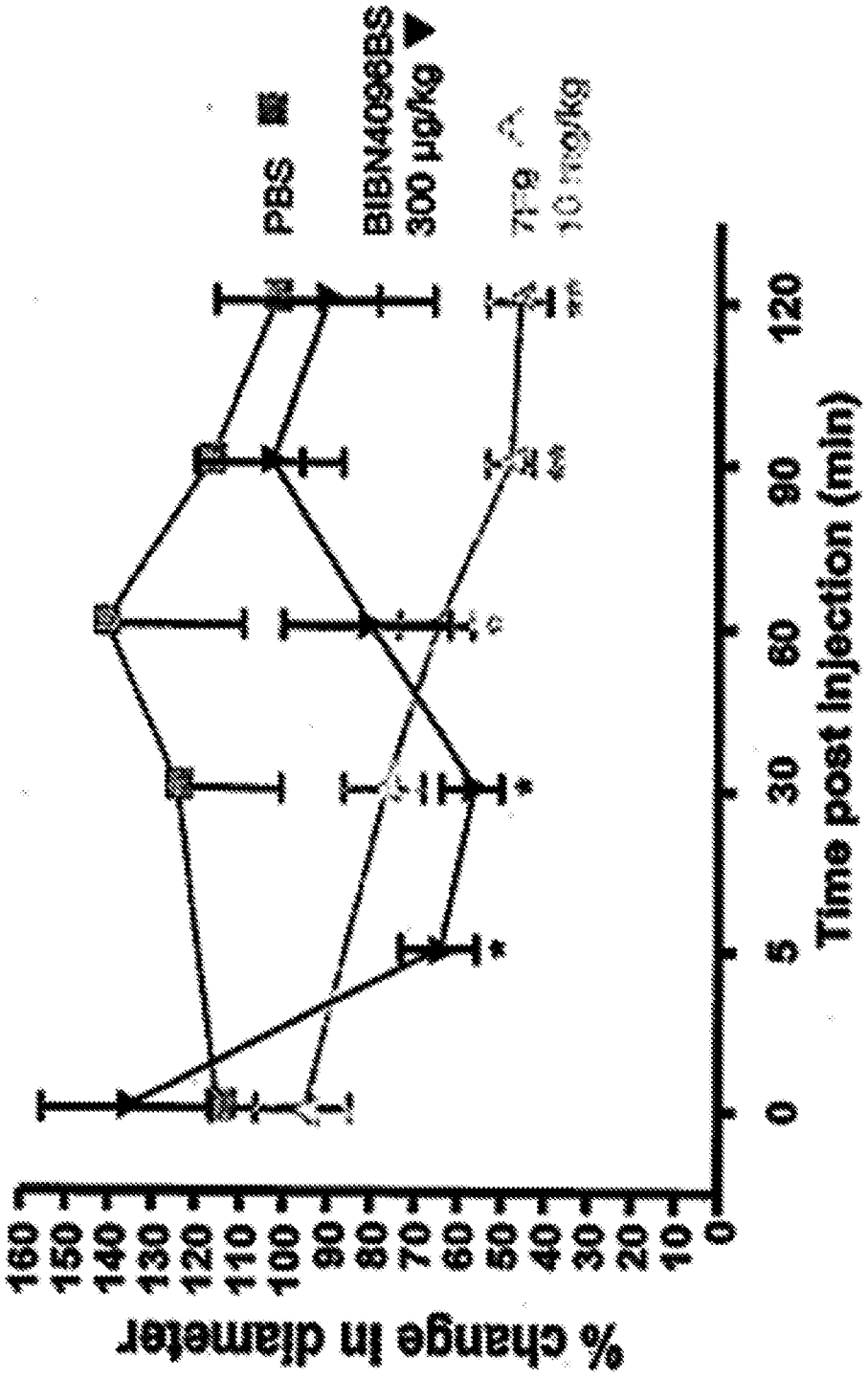

% change in diameter
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
5
30
60
90
120
Time post injection (min)
PBS
BIBN4096BS
300 µg/kg
7TFa
10 mg/kg
*
*

Figure 10

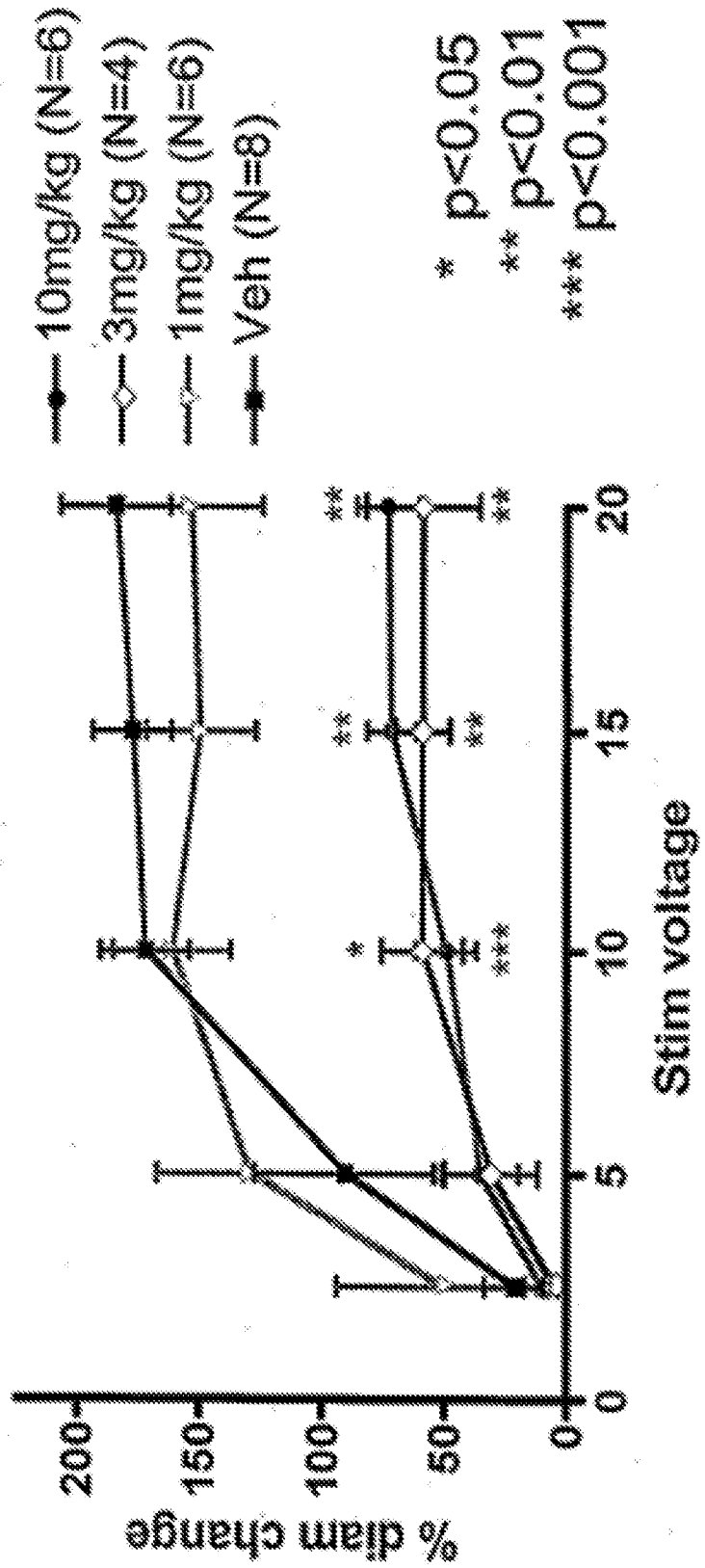

10mg/kg (N=6)
3mg/kg (N=4)
1mg/kg (N=6)
Veh (N=8)
* p<0.05
** p<0.01
*** p<0.001
% diam change
200
150
100
50
0
0
5
10
15
20
Stim voltage

Figure 11A

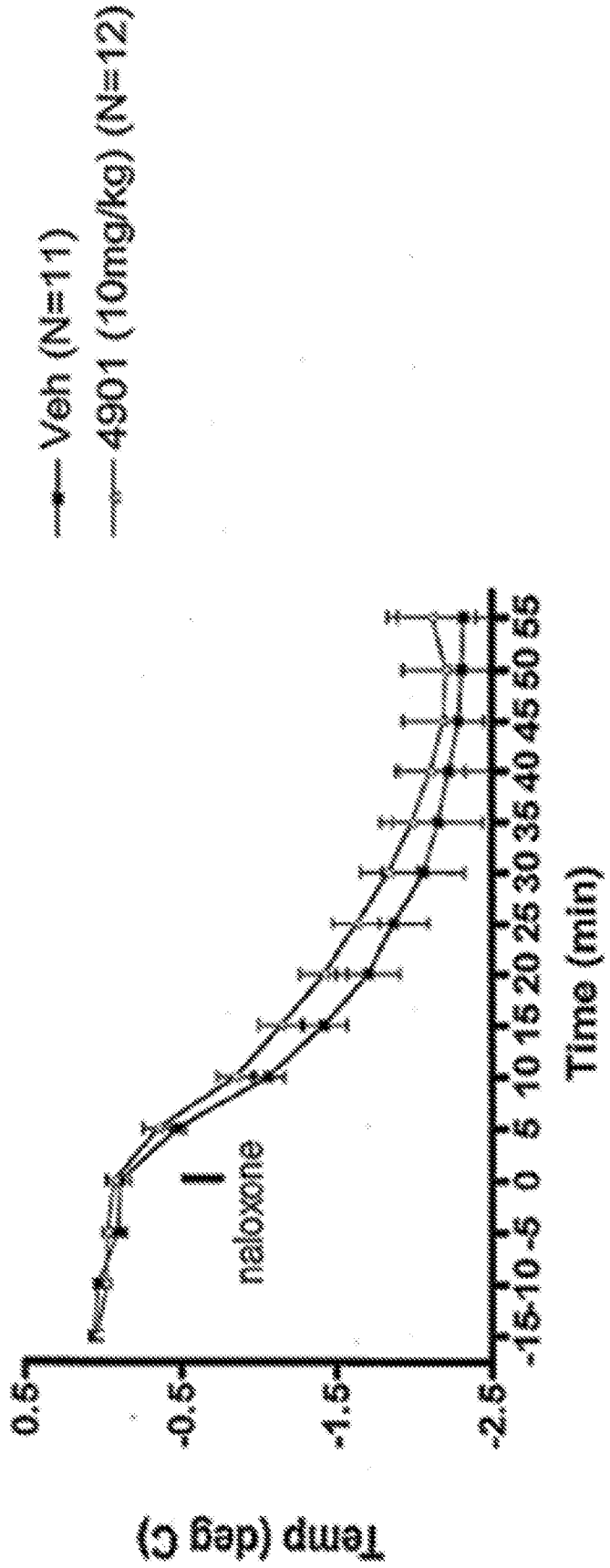
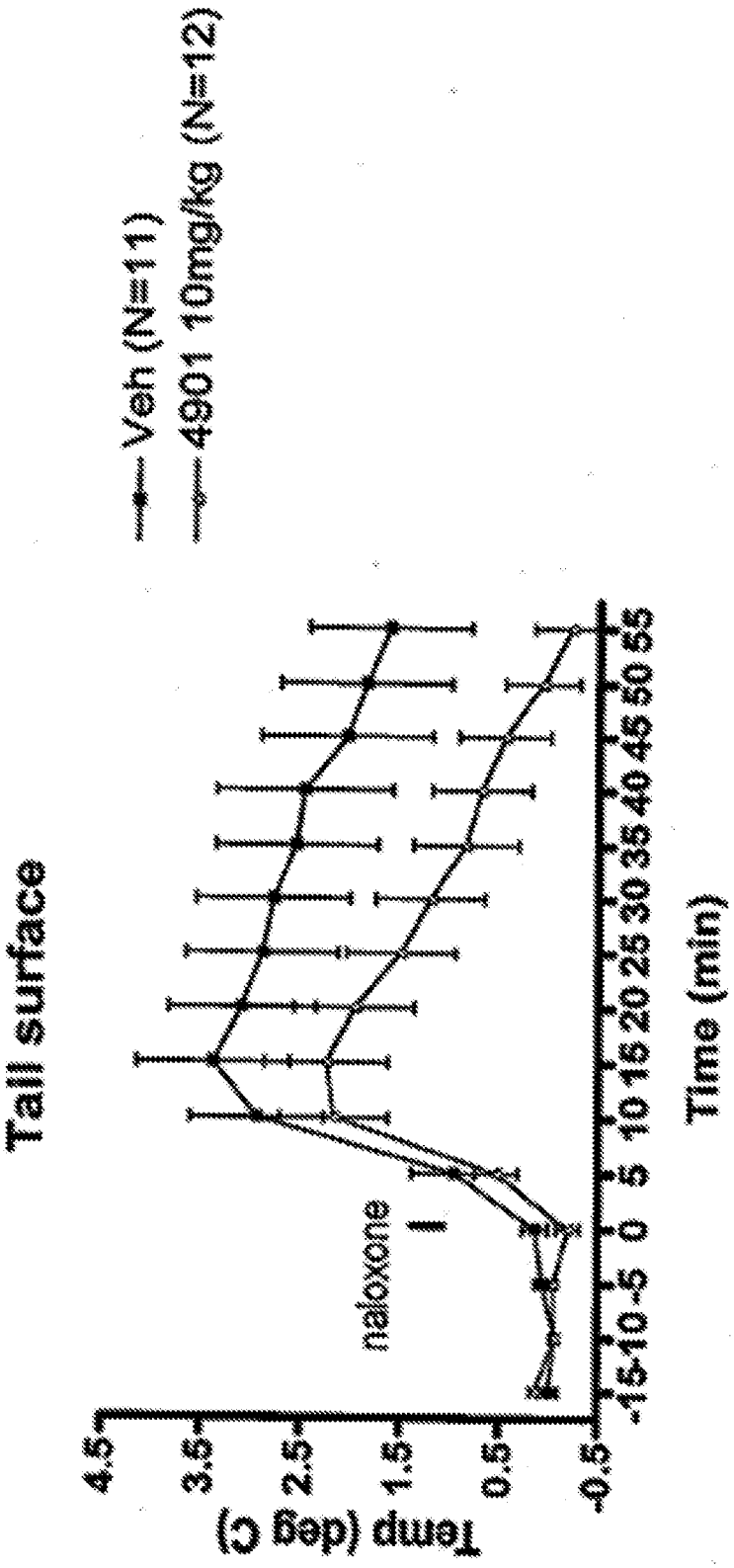
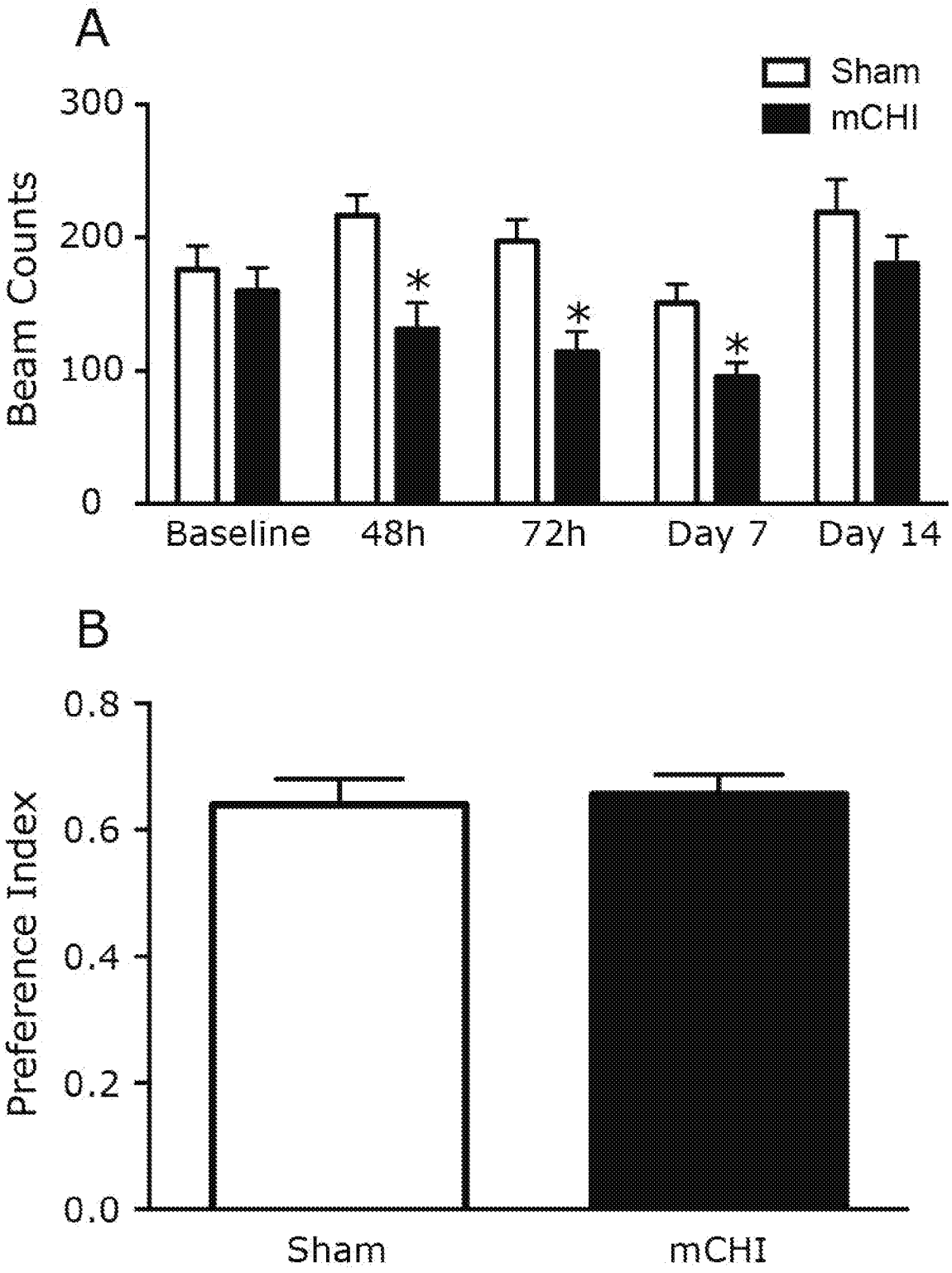
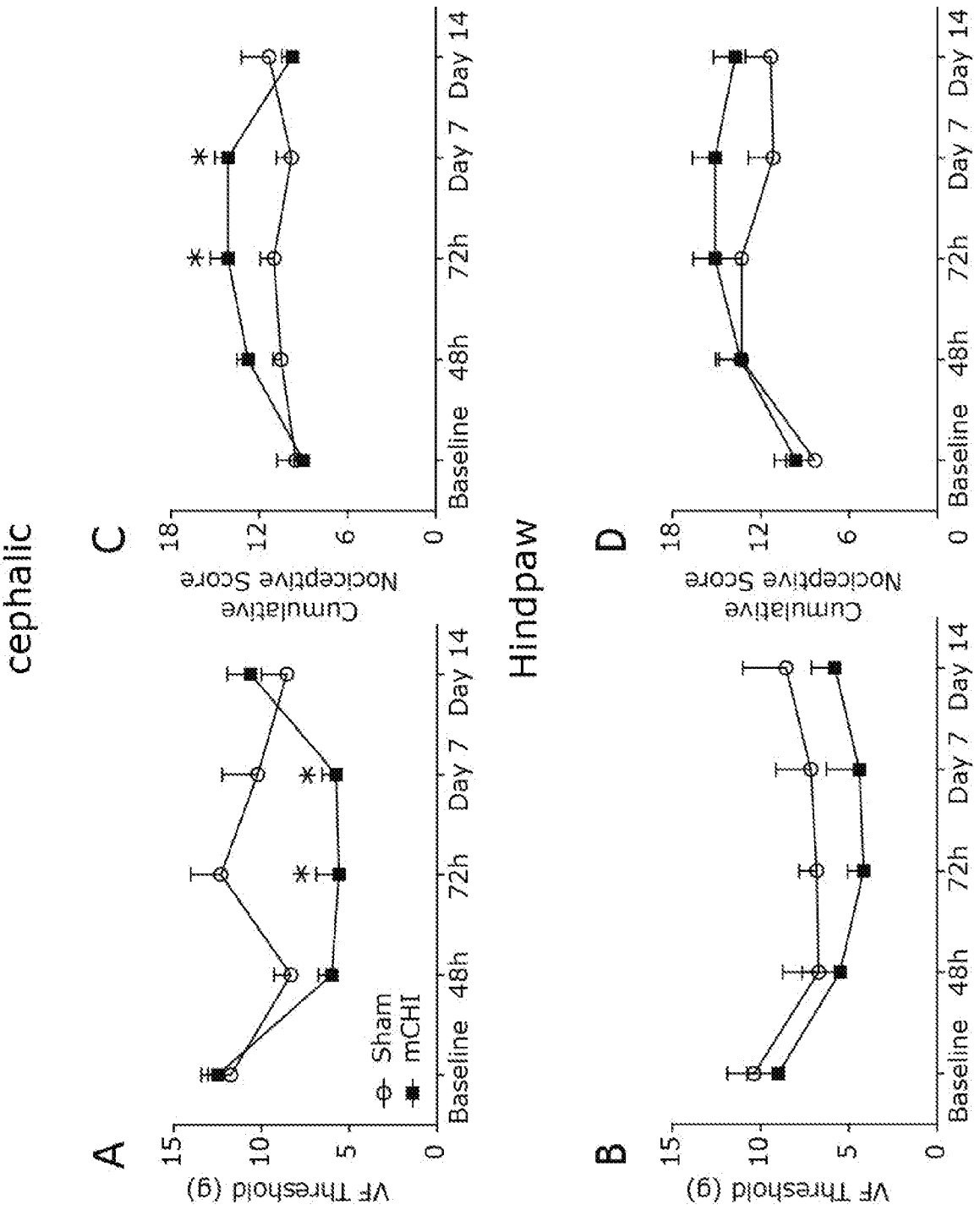

Veh (N=11)
4901 (10mg/kg) (N=12)
Core Temp
naloxone
Temp (deg C)
0.5
-0.5
-1.5
-2.5
-15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55
Time (min)

Figure 11B

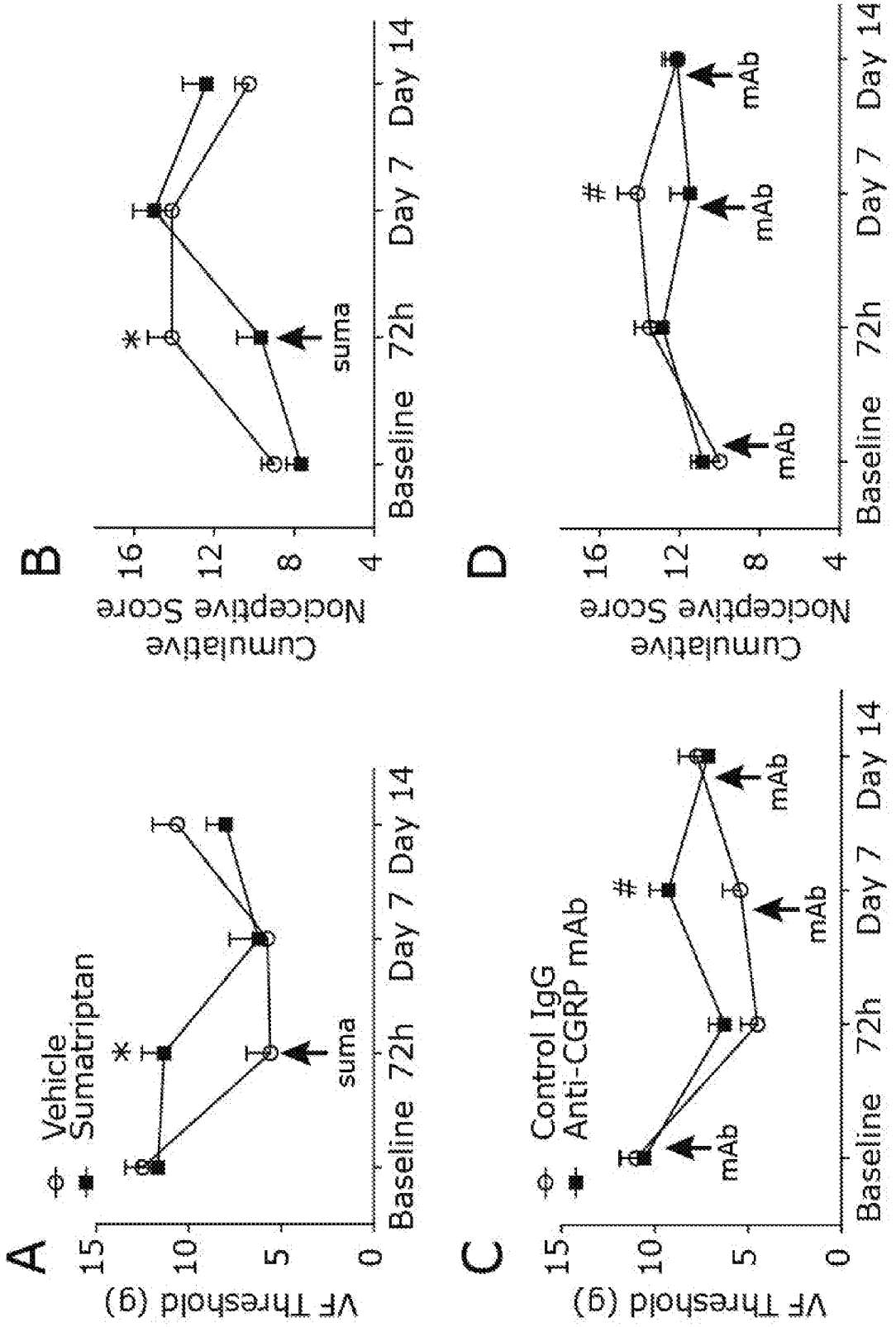

Veh (N=11)
4901 10mg/kg (N=12)
Tail surface
naloxone
Temp (deg C)
4.5
3.5
2.5
1.5
0.5
-0.5
-15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55
Time (min)



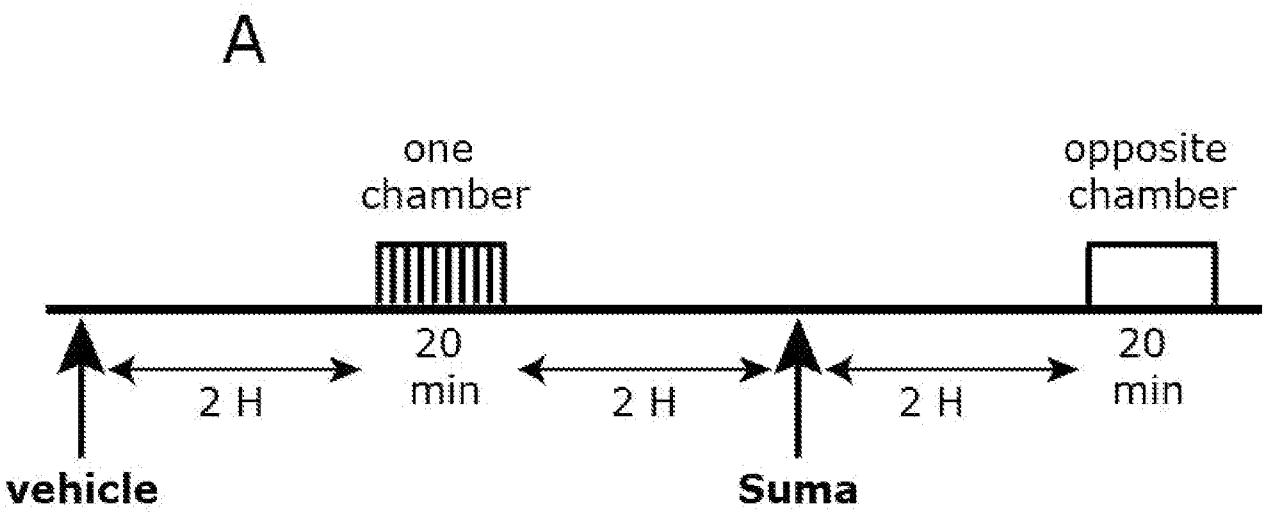
FIGs. 12A and 12B

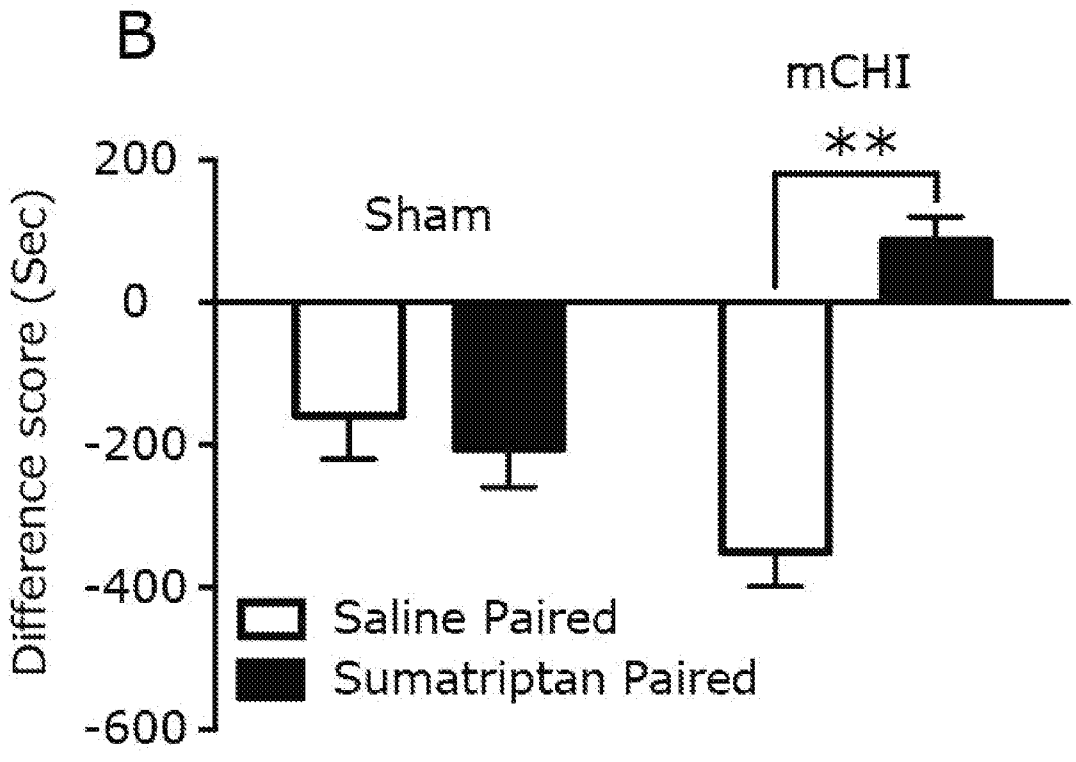


FIGs. 13A to 13D

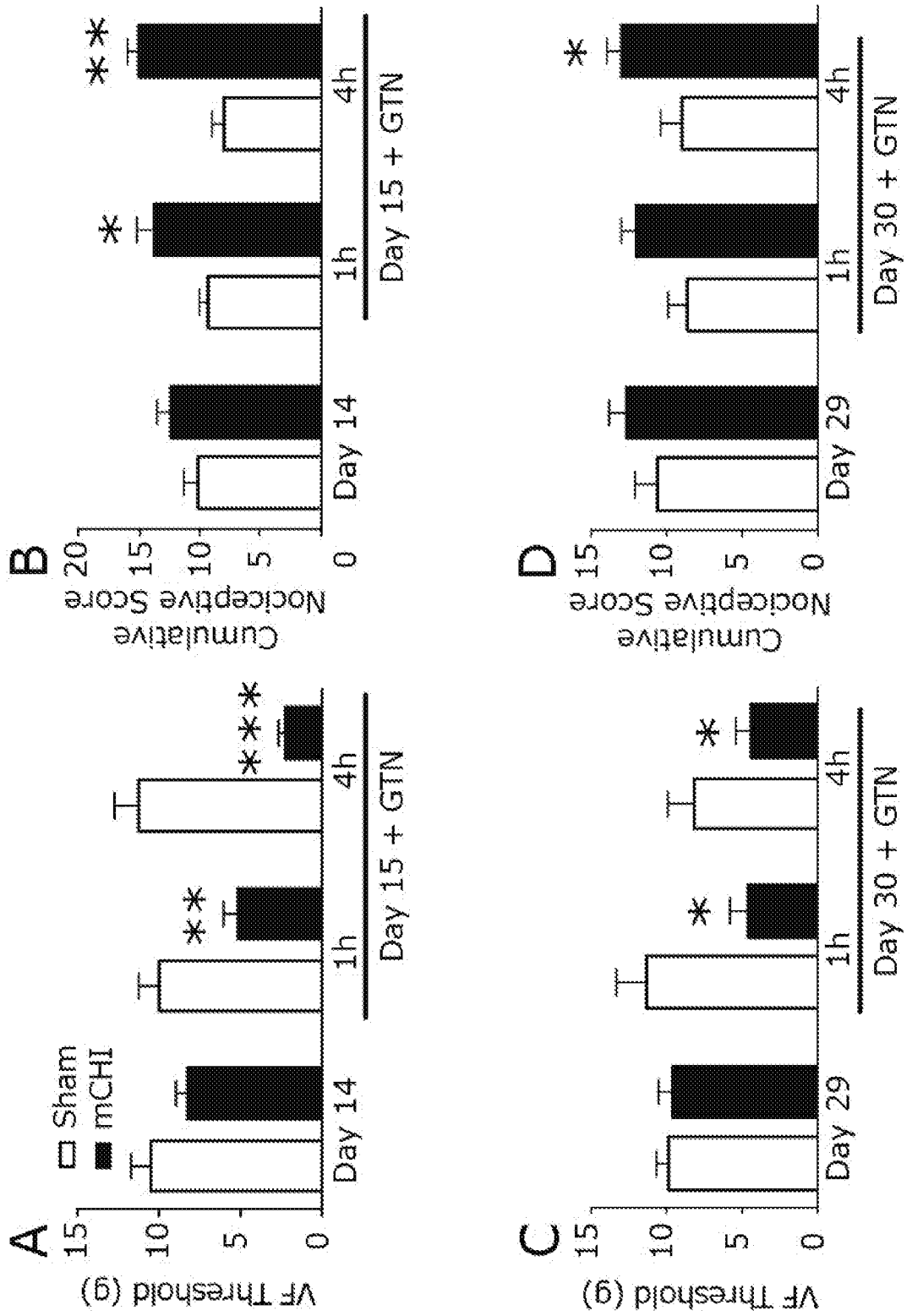


FIGs. 14A to 14D

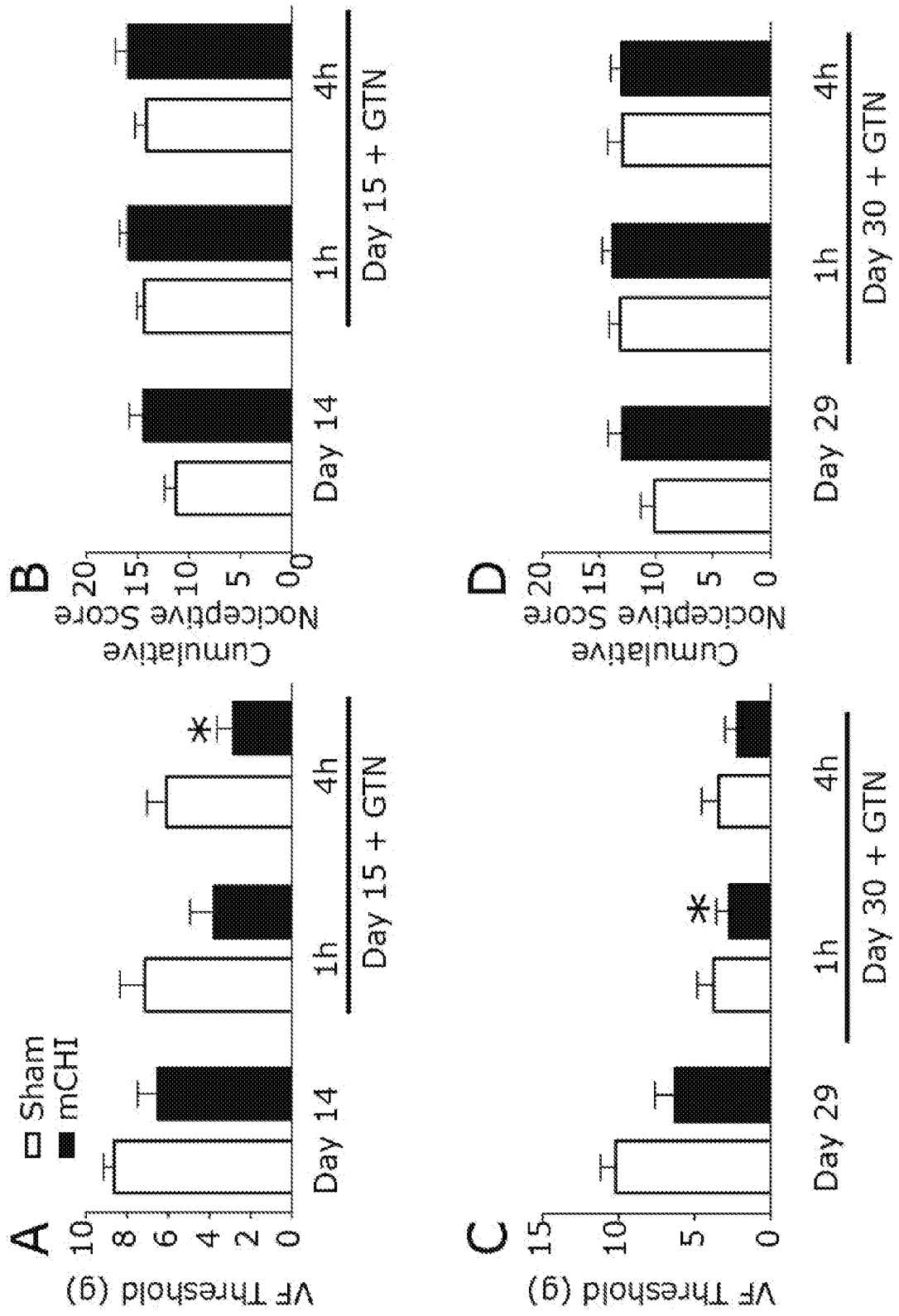




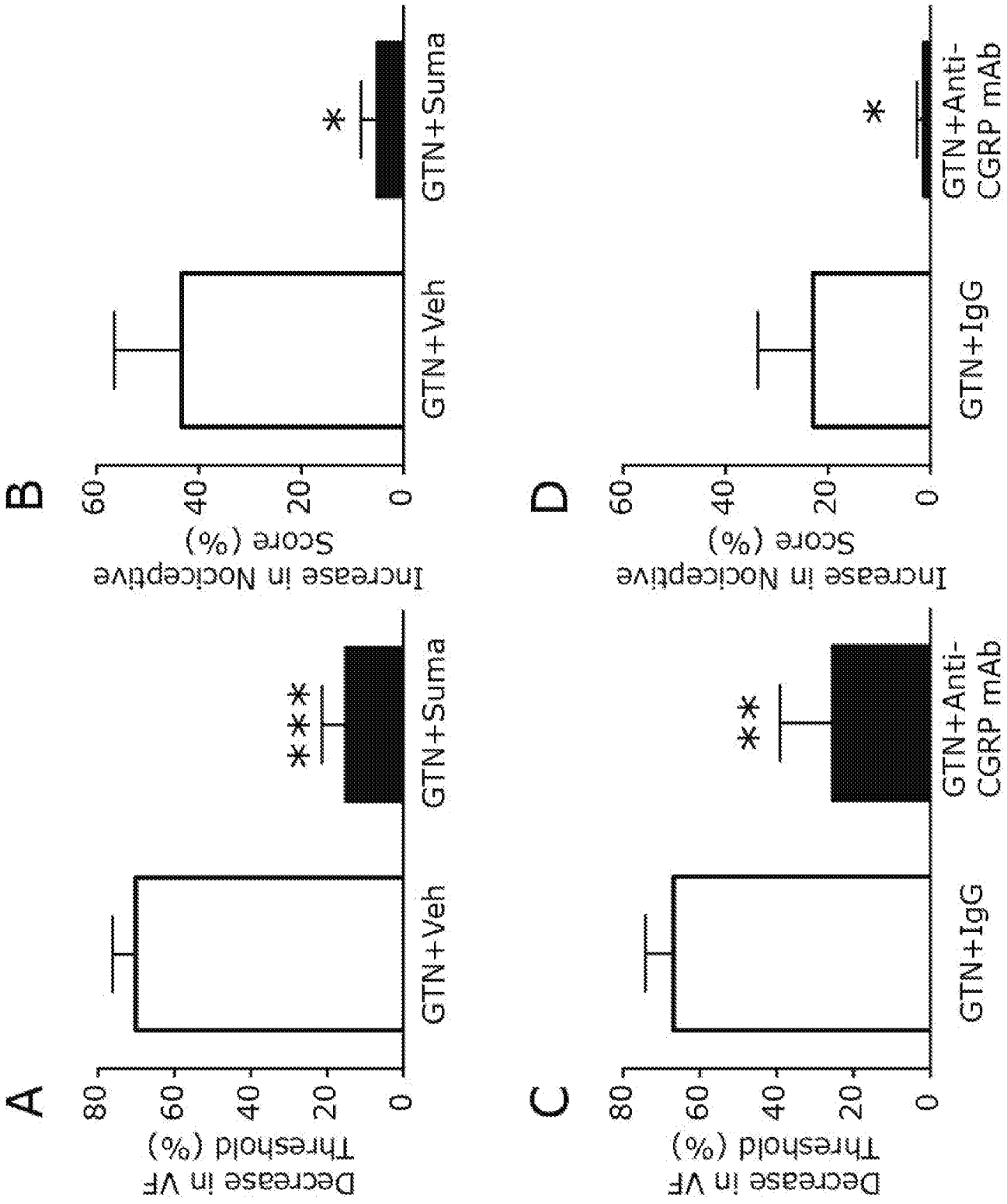
FIGs. 15A and 15B



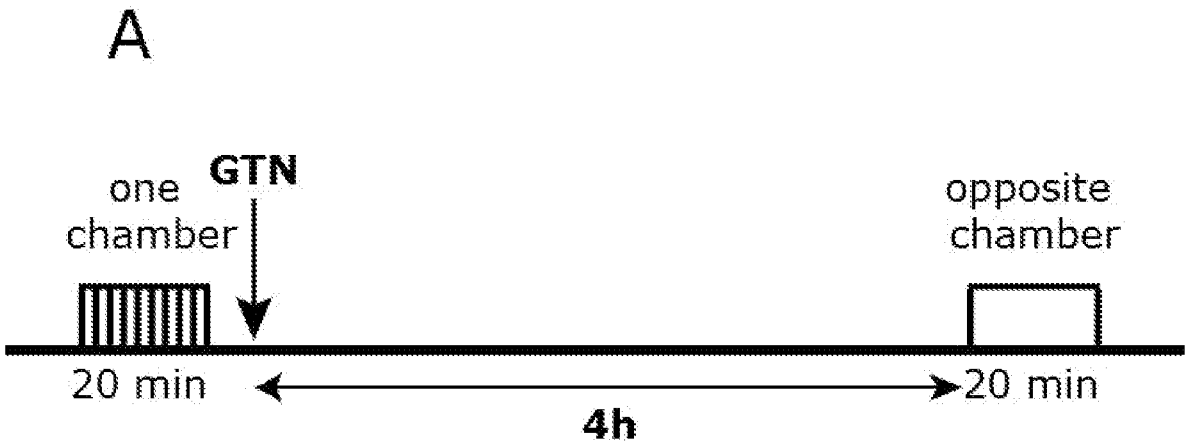
FIGs. 16A to 16D

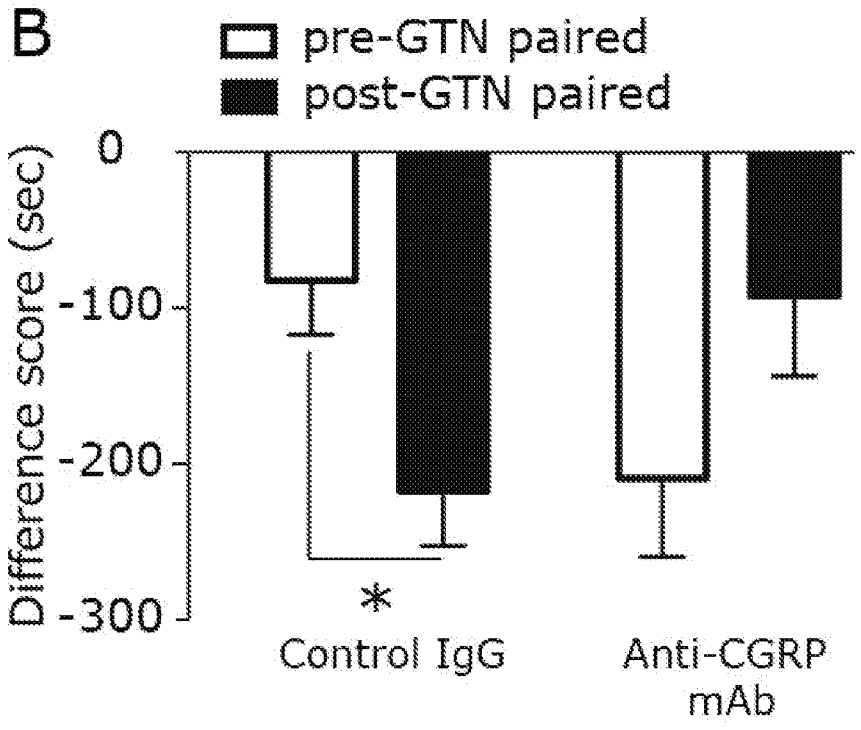


FIGs. 17A to 17D



FIGs. 18A to 18D





FIGs. 19A and 19B

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2016/055720

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 A61K39/395
ADD.

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)
C07K A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MARCELO E. BIGAL ET AL: "Monoclonal Antibodies for Migraine: Preventing Calcitonin Gene-Related Peptide Activity", CNS DRUGS, vol. 28, no. 5, 1 May 2014 (2014-05-01), pages 389-399, XP055328488, AUCKLAND, NZ ISSN: 1172-7047, DOI: 10.1007/s40263-014-0156-4 The whole document, in particular antibody G1 (TEV-48125/LBR-101); sequences 1,2 ----- -/--	1-84

[X] 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

"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
13 December 2016	23/12/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Chapman, Rob

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2016/055720

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.: 1-84(partially)
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:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
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:

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 an 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.:

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/IB2016/055720

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ABBAS ALIBHOY ET AL: "Evaluation of cardiovascular parameters in cynomolgus monkeys following IV administration of LBR-101, a monoclonal antibody against calcitonin gene-related peptide", MABS, vol. 6, no. 4, 1 July 2014 (2014-07-01), pages 12-11, XP055144805, ISSN: 1942-0862, DOI: 10.4161/mabs.29242 Antibody TEV-48125/LBR-101	1-84
Y	Miriam E Tucker: "New Data on CGRP Monoclonal Antibodies for Migraine Prevention", , 23 July 2015 (2015-07-23), XP055328484, Retrieved from the Internet: URL:http://www.medscape.com/viewarticle/846893_print [retrieved on 2016-12-13] Antibody TEV-48125/LBR-101	1-84
Y	MARCELO E. BIGAL ET AL: "Therapeutic antibodies against CGRP or its receptor", BRITISH JOURNAL OF CLINICAL PHARMACOLOGY., vol. 79, no. 6, 22 June 2015 (2015-06-22), pages 886-895, XP055328523, GB ISSN: 0306-5251, DOI: 10.1111/bcp.12591 Antibody TEV-48125/LBR-101	1-84
Y	B V Daiutolo ET AL: "Cortical Injury Modulates the Pain Pathway Partially through Inducible Nitric Oxide Synthase", Department of Neurosurgery Faculty Papers. Paper 65., 1 July 2014 (2014-07-01), XP055328792, Retrieved from the Internet: URL:http://jdc.jefferson.edu/cgi/viewcontent.cgi?article=1065&context=neurosurgeryfp [retrieved on 2016-12-13] Conclusions	1-84
T	D. BREE ET AL: "Development of CGRP-dependent pain and headache related behaviours in a rat model of concussion: Implications for mechanisms of post-traumatic headache", CEPHALALGIA, 29 November 2016 (2016-11-29), XP055328775, GB ISSN: 0333-1024, DOI: 10.1177/0333102416681571 the whole document	

Continuation of Box II.2

Claims Nos.: 1-84(partially)

The application comprises 84 claims referring to a six separate methods of treatment of post-traumatic headache using an anti-CGRP antibody. The number of claims would appear to be unreasonable in consideration of the nature of the invention claimed, i.e. a single method of treatment (Rule 6.1(a) PCT).
Given the undue burden of effort of search for all aspects of the different methods claimed, the ISA has limited the scope of the search to the common linking feature of all claims, i.e. anti-CGRP for use in the treatment of post-traumatic headache.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) declaration be overcome.

(19)中华人民共和国国家知识产权局

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(71)申请人 泰瓦制药国际有限公司
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代理人 郑斌 彭鲲鹏

(51)Int.Cl.
C07K 16/18(2006.01)
A61K 39/395(2006.01)

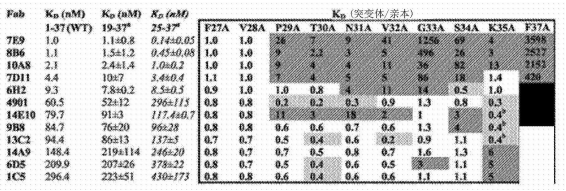
权利要求书4页 说明书112页 附图24页

(54)发明名称

预防、治疗和减轻(持续性)创伤后头痛

(57)摘要

本文中公开了在对象中治疗创伤后头痛和/或与创伤后头痛相关的至少一种继发症状或降低其发病率的方法,其包括向所述对象施用调节CGRP途径的单克隆抗体。还提供了用于所公开方法的组合物。还描述了针对CGRP的拮抗剂抗体G1及来自于G1的抗体。



Fab	K_D (nM)	K_D (nM)	K_D (nM)	K_D (突变体/亲本)								
	1-37(WT)	19-37*	25-37*	F27A	V28A	P29A	T30A	N31A	V32A	G33A	S34A	K35A	F37A
7E9	1.0	1.1±0.8	*0.14±0.05*	1.0	1.0	26	7	9	41	1256	69	4	3998
8B6	1.1	1.5±1.2	*0.45±0.08*	1.0	1.0	9	2.3	3	5	496	26	3	2527
10A8	2.1	2.4±1.4	*1.0±0.2*	1.0	1.0	9	4	4	11	36	82	13	2153
7D11	4.4	10±7	*3.4±0.4*	1.1	1.0	7	4	5	5	86	18	1.4	420
6H2	9.3	7.8±0.2	*8.5±0.5*	0.9	1.0	1.0	0.8	4	11	14	0.5	1.0	
4901	60.5	52±12	*296±115*	0.8	0.8	0.2	0.2	0.3	0.9	1.3	0.8	0.3	
14E10	79.7	91±3	*117.4±0.7*	0.8	0.8	11	3	18	2	1	3	0.4^	
9B8	84.7	76±20	*96±28*	0.8	0.8	0.6	0.6	0.7	0.6	1.3	4	0.4^	
13C2	94.4	86±13	*137±5*	0.7	0.7	0.5	0.4	0.6	0.2	0.9	1.1	0.4^	
14A9	148.4	219±114	*246±20*	0.8	0.7	0.7	0.5	0.8	0.7	1.6	1.3	5	
6D5	209.9	207±26	*378±22*	0.8	0.7	0.5	0.4	0.6	0.5	3	1.1	5	
1C5	296.4	223±51	*430±173*	0.8	0.8	0.6	0.4	0.6	0.6	1.1	1.1	5	

CN 108473560 A

1.包含调节CGRP途径的单克隆抗体的组合物,其用于在对象中治疗创伤后头痛或降低其发病率。

2.包含调节CGRP途径的单克隆抗体的组合物,其用于在对象中治疗与创伤后头痛相关的至少一种继发症状或降低其发病率。

3.权利要求1或2所述的组合物,其中所述组合物包含约225mg至约1000mg的所述单克隆抗体。

4.前述权利要求中任一项所述的组合物,其中所述组合物每月施用。

5.权利要求4所述的组合物,其中所述组合物每月施用持续三个月或更久。

6.权利要求4或5所述的组合物,其中向所述对象施用的所述单克隆抗体的剂量为约225mg至约675mg。

7.权利要求1至4中任一项所述的组合物,其中所述组合物作为单次剂量施用。

8.权利要求7所述的组合物,其中向所述对象施用的所述单克隆抗体的剂量为约675mg至约1000mg。

9.前述权利要求中任一项所述的组合物,其中所述治疗或降低包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、降低6项头痛影响测试(HIT-6)失能评分、或其任意组合。

10.权利要求9所述的组合物,其中相对于所述对象中的施用前水平,每月头痛日数降低至少约50%。

11.前述权利要求中任一项所述的组合物,其中所述单克隆抗体是抗CGRP拮抗剂抗体。

12.前述权利要求中任一项所述的组合物,其中所述组合物皮下或静脉内施用。

13.前述权利要求中任一项所述的组合物,其中所述组合物利用包含一定剂量所述单克隆抗体的预填充注射器进行施用。

14.前述权利要求中任一项所述的组合物,其中所述单克隆抗体以至少150mg/mL的浓度配制。

15.前述权利要求中任一项所述的组合物,其中所述单克隆抗体以小于2mL的体积施用。

16.前述权利要求中任一项所述的组合物,其中所述组合物以第二药剂与所述单克隆抗体同时或先后的方式施用于所述对象。

17.权利要求16所述的组合物,其中在施用所述单克隆抗体之后,所述对象对所述第二药剂的每月使用降低至少15%。

18.前述权利要求中任一项所述的组合物,其中所述对象是人。

19.前述权利要求中任一项所述的组合物,其中所述单克隆抗体是人的或人源化的。

20.前述权利要求中任一项所述的组合物,其中所述单克隆抗体包含:(a)具有SEQ ID NO:3所示CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6所示CDR L1、SEQ ID NO:7所示CDR L2和SEQ ID NO:8所示CDR L3的抗体;或(b)如表6中所示的根据(a)的抗体的变体。

21.包含治疗有效量的调节降钙素基因相关肽(CGRP)途径的单克隆抗体的组合物,其用于在已遭受轻度创伤性脑损伤的对象中预防、治疗创伤后头痛或降低其发病率。

22.权利要求21所述的组合物,其中所述单克隆抗体静脉内或皮下施用于所述对象。

23.权利要求21所述的组合物，其中所述单克隆抗体以约1小时的输注静脉内施用于所述对象。

24.权利要求21所述的组合物，其中所述组合物的一个或更多个另外的剂量施用于所述对象。

25.权利要求21所述的组合物，其中所述单克隆抗体以约675mg的剂量施用。

26.权利要求25所述的组合物，其中所述单克隆抗体在随后的两个月以约225mg的剂量施用于所述对象。

27.权利要求21所述的组合物，其中所述单克隆抗体以约900mg的剂量施用。

28.权利要求27所述的组合物，其中所述单克隆抗体以约900mg的剂量每季度施用。

29.权利要求21所述的组合物，其中所述单克隆抗体在所述脑损伤之后立即施用。

30.权利要求21所述的组合物，其中所述治疗或降低包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、减轻颈部疼痛、降低6项头痛影响测试(HIT-6)失能评分、改善12项简式健康调查(SF-12)评分、改善患者变化总体印象(PGIC)量表、改善运动震荡评估工具3(SCAT-3)评分、或其任意组合。

31.权利要求21所述的组合物，其中所述抗体从包含一定剂量所述单克隆抗体的预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器施用于所述对象。

32.权利要求21所述的组合物，其中所述单克隆抗体作为包含浓度为至少约150mg/mL的所述抗体的制剂施用。

33.权利要求21所述的组合物，其中所述单克隆抗体以小于2mL的体积施用。

34.权利要求21所述的组合物，其中所述组合物以第二药剂与所述单克隆抗体同时或先后的方式施用于所述对象。

35.权利要求34所述的组合物，其中所述第二药剂选自：5-HT1激动剂、曲普坦类、阿片类、β-肾上腺素能拮抗剂、麦角生物碱类和非甾体抗炎药(NSAID)。

36.权利要求35所述的组合物，其中所述曲普坦类是舒马普坦、佐米曲普坦、那拉曲普坦、利扎曲普坦、依来曲普坦、阿莫曲普坦或夫罗曲普坦。

37.权利要求21所述的组合物，其中所述单克隆抗体是抗CGRP拮抗剂抗体。

38.权利要求21所述的组合物，其中所述单克隆抗体是人的或人源化的。

39.权利要求21所述的组合物，其中所述单克隆抗体是人源化抗CGRP拮抗剂抗体。

40.权利要求21所述的组合物，其中所述单克隆抗体包含SEQ ID NO:3所示CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6所示CDR L1、SEQ ID NO:7所示CDR L2和SEQ ID NO:8所示CDR L3。

41.权利要求21所述的组合物，其中所述单克隆抗体是IgG1、IgG2、IgG3或IgG4抗体。

42.权利要求21所述的组合物，其中所述对象是人。

43.在对象中治疗创伤后头痛或降低其发病率的方法，其包括向所述对象施用调节CGRP途径的单克隆抗体。

44.在对象中治疗与创伤后头痛相关的至少一种继发症状或降低其发病率的方法，其包括向所述对象施用调节CGRP途径的单克隆抗体。

45.权利要求43或44所述的方法，其中向所述对象施用的所述单克隆抗体的量为约225mg至约1000mg。

46.前述权利要求中任一项所述的方法,其中所述单克隆抗体每月施用。

47.权利要求46所述的方法,其中所述单克隆抗体每月施用持续三个月或更久。

48.权利要求46或47所述的方法,其中向所述对象施用的所述单克隆抗体的剂量为约225mg至约675mg。

49.权利要求43至46中任一项所述的方法,其中所述单克隆抗体作为单次剂量施用。

50.权利要求49所述的方法,其中向所述对象施用的所述单克隆抗体的剂量为约675mg至约1000mg。

51.前述权利要求中任一项所述的方法,其中所述治疗或降低包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、降低6项头痛影响测试(HIT-6)失能评分、或其任意组合。

52.权利要求51所述的方法,其中相对于所述对象中的施用前水平,每月头痛日数降低至少约50%。

53.前述权利要求中任一项所述的方法,其中所述单克隆抗体是抗CGRP拮抗剂抗体。

54.前述权利要求中任一项所述的方法,其中所述施用是皮下或静脉内施用。

55.前述权利要求中任一项所述的方法,其中所述施用包括利用包含一定剂量所述单克隆抗体的预填充注射器。

56.前述权利要求中任一项所述的方法,其中所述单克隆抗体以至少150mg/mL的浓度配制。

57.前述权利要求中任一项所述的方法,其中所述单克隆抗体以小于2mL的体积施用。

58.前述权利要求中任一项所述的方法,其包括与所述单克隆抗体同时或先后向所述对象施用第二药剂。

59.权利要求58所述的方法,其中在施用所述单克隆抗体之后,所述对象对所述第二药剂的每月使用降低至少15%。

60.前述权利要求中任一项所述的方法,其中所述对象是人。

61.前述权利要求中任一项所述的方法,其中所述单克隆抗体是人的或入源化的。

62.前述权利要求中任一项所述的方法,其中所述单克隆抗体包含:(a)具有SEQ ID NO:3所示CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6所示CDR L1、SEQ ID NO:7所示CDR L2和SEQ ID NO:8所示CDR L3的抗体;或(b)如表6中所示的根据(a)的抗体的变体。

63.在对象中预防、治疗创伤后头痛或降低其发病率的方法,所述方法包括:

选择已遭受轻度创伤性脑损伤的对象;以及

向所述对象施用治疗有效量的调节降钙素基因相关肽(CGRP)途径的单克隆抗体。

64.权利要求63所述的方法,其中所述单克隆抗体静脉内或皮下施用于所述对象。

65.权利要求63所述的方法,其中所述单克隆抗体以约1小时的输注静脉内施用于所述对象。

66.权利要求63所述的方法,其还包括向所述对象施用一个或更多个另外剂量的所述单克隆抗体。

67.权利要求63所述的方法,其中所述单克隆抗体以约675mg的剂量施用。

68.权利要求67所述的方法,其还包括在随后的两个月以约225mg的剂量向所述对象施

用所述单克隆抗体。

69.权利要求63所述的方法，其中所述单克隆抗体以约900mg的剂量施用。

70.权利要求69所述的方法，其中所述单克隆抗体以约900mg的剂量每季度施用。

71.权利要求63所述的方法，其中所述单克隆抗体在所述脑损伤之后立即施用。

72.权利要求63所述的方法，其中所述治疗或降低包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、减轻颈部疼痛、降低6项头痛影响测试(HIT-6)失能评分、改善12项简式健康调查(SF-12)评分、改善患者变化总体印象(PGIC)量表、改善运动震荡评估工具3(SCAT-3)评分、或其任意组合。

73.权利要求63所述的方法，其中所述施用包括从包含一定剂量所述单克隆抗体的预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器将所述抗体施用于所述对象。

74.权利要求63所述的方法，其中所述单克隆抗体作为包含浓度为至少约150mg/mL的所述抗体的制剂施用。

75.权利要求63所述的方法，其中所述单克隆抗体以小于2mL的体积施用。

76.权利要求63所述的方法，其还包括与所述单克隆抗体同时或先后向所述对象施用第二药剂。

77.权利要求76所述的方法，其中所述第二药剂选自:5-HT1激动剂、曲普坦类、阿片类、β-肾上腺素能拮抗剂、麦角生物碱类和非甾体抗炎药(NSAID)。

78.权利要求77所述的方法，其中所述曲普坦类是舒马普坦、佐米曲普坦、那拉曲普坦、利扎曲普坦、依来曲普坦、阿莫曲普坦或夫罗曲普坦。

79.权利要求63所述的方法，其中所述单克隆抗体是抗CGRP拮抗剂抗体。

80.权利要求63所述的方法，其中所述单克隆抗体是人的或人源化的。

81.权利要求63所述的方法，其中所述单克隆抗体是人源化抗CGRP拮抗剂抗体。

82.权利要求63所述的方法，其中所述单克隆抗体包含SEQ ID NO:3所示CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6所示CDR L1、SEQ ID NO:7所示CDR L2和SEQ ID NO:8所示CDR L3。

83.权利要求63所述的方法，其中所述单克隆抗体是IgG1、IgG2、IgG3或IgG4抗体。

84.权利要求63所述的方法，其中所述对象是人。

预防、治疗和减轻(持续性)创伤后头痛

[0001] 相关申请的交叉引用

[0002] 本申请要求于2015年9月24日提交的美国申请No.62/232,343和于 2016年8月16日提交的美国申请No.62/375,825的优先权权益。在先申请的内容在此通过引用整体并入。

背景技术

[0003] 降钙素基因相关肽(calcitonin gene-related peptide,CGRP)是37个氨基酸的神经肽,其属于包括降钙素、肾上腺髓质素、肾上腺髓质素2(垂体中叶素(intermedin))和胰淀素的肽家族(Russell等,Physiol Rev 94:1099-1142,2014)。在人中,存在两种形式的CGRP(α-CGRP和β-CGRP) 并且其具有类似的活性。其因三个氨基酸而不同并且表现出不同的分布。至少两种CGRP受体亚型也可解释差异活性。CGRP是中枢神经系统中的神经递质,并且已经显示其在外周中是强效的血管扩张剂,其中包含 CGRP的神经元与血管密切相关。CGRP介导的血管扩张作为导致血浆外渗和微血管系统血管扩张的事件级联的一部分还与神经源性炎症相关。

[0004] 头痛是创伤性脑损伤(traumatic brain injury,TBI)的常见并发症,在(反复性)轻度TBI(mild TBI,mTBI)或轻度闭合性头部损伤(mild closed head injury,mCHI)之后尤其如此。这些头痛的管理可具有挑战性。头痛的频率可较高(如果不是每天的话)。认识到创伤后头痛(post-traumatic headache,PTH)的复杂性,国际头痛协会(International Headache Society)在国际头痛疾病分类(International Classification of Headache Disorder,ICHD)中具有这些头痛特定的单独分类(Headache Classification Subcommittee of the International Headache Society,2004,以及更新的版本,2013)。参见Riechers等(Handbook of Clinical Neurology 128:567-78,2015)。第二版(ICHD-II)和第三版(ICHD-3β)将PTH定义为在创伤事件或出现昏迷状态7天内发生的头痛。在损伤之后的前三个月内,这些头痛可被定义为急性的;然而,如果其持续下去超过该时间,则将其定义为慢性的。还可使用头部损伤的严重程度来将PTH的类别细分为由轻度头部损伤引起的PTH(格拉斯哥昏迷量表评分(Glasgow Coma Scale score,GCS)13-15,意识丧失短于30分钟或其他震荡症状)或由严重头部损伤引起的PTH(GCS小于13,意识丧失超过30分钟,健忘超过48小时,或异常成像)。添加至ICHD-II的另一个诊断类别是由于颅内血肿引起的头痛,其中硬膜外和硬膜下血肿是主要考虑的血肿。为了符合 ICHD标准,必须在成像时看到这些血肿,并且在硬膜外血肿的情况下,其必须在血肿24小时内发生,并在外科手术干预后3个月内消退,而硬膜下血肿性头痛应在24至72小时内发生并且不存在具体的消退标准。

[0005] 发明概述

[0006] 本文中公开了抗CGRP拮抗剂抗体以及使用所述抗CGRP拮抗剂抗体来预防、治疗(持续性)创伤后头痛或降低其发病率的方法。本文还公开了在对象中预防、治疗(持续性)创伤后头痛或降低其发病率的方法,其包括向对象施用调节CGRP途径的单克隆抗体。

[0007] 还提供了在对象中预防、治疗与(持续性)创伤后头痛相关的至少一种继发症状或降低其发病率的方法,其包括向对象施用调节CGRP途径的单克隆抗体。在一些实施方案中,

施用于患者的单克隆抗体的量可以是约 225mg至约1000mg，例如约675mg或约900mg。因此，在一些方面，在对象中预防、治疗(持续性)创伤后头痛或降低其发病率的方法可包括向对象施用调节CGRP途径的单克隆抗体，其中施用于患者的单克隆抗体的量可以是约225mg至约1000mg，例如约675mg或约900mg。在另一些方面，在对象中预防、治疗与(持续性)创伤后头痛相关的至少一种继发症状或降低其发病率的方法可包括向对象施用调节CGRP途径的单克隆抗体，其中施用于患者的单克隆抗体的量可以是约225mg至约1000 mg，例如约675mg或约900mg。在一个实施方案中，给药方案包括皮下施用约675mg的初始抗体剂量，然后皮下施用约225mg的每月抗体剂量持续约2个月，例如约3个月、4个月、5个月、6个月或12个月。另一种给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量，随后每季度以约60分钟的输注静脉内施用约900mg的剂量持续约1年，例如2年、3年、4年或5年。

[0008] 合适的施用方案包括但不限于每月、每季度或单次剂量。在一些实施方案中，单克隆抗体可以每月施用。例如，单克隆抗体可每月施用持续1、 2、3、4、5、6、7、8、9、10、11、12个月或更久。在一些方面，单克隆抗体可每月施用持续3个月或更久。当每月施用时，施用于患者的单克隆抗体的剂量可以是约225mg至约900mg。

[0009] 单克隆抗体可作为单次剂量施用。当作为单次剂量施用时，施用于患者的单克隆抗体的剂量可以是约675mg至约1000mg。

[0010] 治疗或降低可包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、降低6项头痛影响测试(6-item Headache Impact Test，HIT-6)失能评分、改善12项简式健康调查(12-Item Short Form Health Survey，SF-12)评分(Ware等，Med Care 4:220-233，1996)、降低患者变化总体印象(Patient Global Impression of Change，PGIC)评分(Hurst等，J Manipulative Physiol Ther 27:26-35，2004)、改善运动震荡评估工具3(Sport ConCuSSion ASSeSment tool 3，SCAT-3) 评分(McCrory等British Journal of Spots Medicine 47:263-266，2013)、或其任意组合。在一些实施方案中，在单次施用之后，可降低每月头痛日数至少七天。

[0011] 在一些实施方案中，相对于对象中的施用前水平，对象在所述施用之后经历的每月头痛小时降低40个或更多个小时(例如45、50、55、60、 65、70、75、80或更多)。每月头痛小时数可降低超过60个小时。在一些实施方案中，相对于对象中的施用前水平，对象在所述施用之后经历的每月头痛小时降低25％或更多(例如，30％、35％、40％、45％、50％或更多)。每月头痛小时数可降低40％或更多。在一些实施方案中，相对于对象中的施用前水平，对象在所述施用之后经历的每月头痛日数降低三天或更多(例如，3、4、5、6、7、8、9、10、11、12、13、14、15、16、 17、18、19、20天或更多)。在一些实施方案中，相对于对象中的施用前水平，每月头痛日数可降低至少约50％。因此，在一些方面，每月头痛日数可降低至少约50％、至少约55％、至少约60％、至少约65％、至少约 70％、至少约75％、至少约80％或至少约90％。

[0012] 在一些实施方案中，施用可以是皮下施用。在一些实施方案中，施用可以是静脉内施用。在一些实施方案中，施用可包括利用包含一定剂量单克隆抗体的预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器。在一些实施方案中，单克隆抗体可以以至少150mg/mL的浓度配制。在一些实施方案中，单克隆抗体可以以小于2mL(例如约1.5mL)的体积施用。

[0013] 在一些实施方案中，所述方法还包括与单克隆抗体同时或先后向所述对象施用第

二药剂。第二药剂可以是5-HT1激动剂、曲普坦类(triptan)、麦角生物碱类和非甾体抗炎药中的任一种。在一些实施方案中,第二药剂是对象预防性摄取的药剂。在一些实施方案中,在施用单克隆抗体之后,对象对第二药剂的每月使用降低至少约15%,例如至少16%、17%、18%、20%、22%、25%、28%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或至少约95%。在一些实施方案中,第二药剂是曲普坦类。

[0014] 在一些实施方案中,对象是人。

[0015] 单克隆抗体可以是抗CGRP拮抗剂抗体。在一些实施方案中,单克隆抗体是人单克隆抗体或人源化单克隆抗体。在一些实施方案中,单克隆抗体包含:(a)具有SEQ ID NO:3所示CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6所示CDR L1、SEQ ID NO:7 所示CDR L2和SEQ ID NO:8所示CDR L3的抗体;或(b)如表6中所示的根据(a)的抗体的变体。

[0016] 还公开了降低患有(持续性)创伤后头痛的对象经历的每月头痛小时数的方法。在一个实施方案中,所述方法包括向所述对象施用一定量的调节CGRP途径的单克隆抗体,其中所述单克隆抗体的量在单次剂量之后有效地将每月头痛小时数降低至少20(例如,25、30、35、40、45、50、55、60、65、70个或更多个头痛小时)。在一些实施方案中,每月头痛小时数降低至少约50个小时。在一个实施方案中,所述方法包括向对象施用一定量的调节CGRP途径的单克隆抗体,其中所述单克隆抗体的量在单次剂量之后有效地将每月头痛小时数降低至少15%(例如20%、25%、30%、35%、40%或更多)。在一些实施方案中,每月头痛小时数降低至少约30%。在一些实施方案中,单克隆抗体是抗CGRP拮抗剂抗体。在一些实施方案中,施用于患者的单克隆抗体的量为约225mg至约1000mg。在一些实施方案中,单克隆抗体每月施用。在一些实施方案中,单克隆抗体作为单次剂量施用。在一些实施方案中,所述施用是皮下施用或静脉内施用。在一些实施方案中,单克隆抗体以至少150mg/mL的浓度配制。在一些实施方案中,单克隆抗体以小于2mL(例如约1.5mL)的体积施用。在一些实施方案中,对象是人。在一些实施方案中,单克隆抗体是人的或人源化的。在一些实施方案中,单克隆抗体包含:(a)具有SEQ ID NO:3所示 CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6所示CDR L1、SEQ ID NO:7所示CDR L2和SEQ ID NO:8所示CDR L3的抗体;或(b)如表6中所示的根据(a)的抗体的变体。

[0017] 还公开了降低患有(持续性)创伤后头痛的对象经历的每月头痛日数的方法。在一个实施方案中,所述方法包括向所述对象施用一定量的调节 CGRP途径的单克隆抗体,其中所述单克隆抗体的量为在单次剂量之后有效地使每月头痛日数降低至少3(例如,3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20个头痛日或更多)。在一些实施方案中,每月头痛日数降低至少约6个头痛日。在一些实施方案中,相对于对象中的施用前水平,每月头痛日数可降低至少约50%。因此,在一些方面,每月头痛日数可降低至少约50%、至少约55%、至少约60%、至少约65%、至少约70%、至少约75%、至少约80%或至少约90%。在一些实施方案中,单克隆抗体是抗CGRP拮抗剂抗体。在一些实施方案中,施用于患者的单克隆抗体的量为约225mg至约1000mg。在一些实施方案中,单克隆抗体每月施用。在一些实施方案中,单克隆抗体作为单次剂量施用。在一些实施方案中,所述施用是皮下或静脉内施用。在一些实施方案中,单克隆抗体以至少150mg/mL的浓度配制。在一些实施方案中,其中单克隆抗体以小于2mL(例如约1.5mL)的体积施用。在一些实施方案中,对象是人。在一些实施方

案中，单克隆抗体是人的或人源化的。在一些实施方案中，单克隆抗体包含：(a)具有SEQ ID NO:3所示CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6 所示CDR L1、SEQ ID NO:7所示CDR L2和SEQ ID NO:8所示CDR L3 的抗体；或(b)如表6中所示的根据(a)的抗体的变体。

[0018] 还公开了在患有(持续性)创伤后头痛的对象中降低任何急性头痛药物的使用的方法，其包括向对象施用调节CGRP途径的单克隆抗体(例如，抗CGRP拮抗剂抗体)，其中所述单克隆抗体的量有效地使对象对抗头痛药物的每月使用降低至少15％(例如，20％、25％、30％、35％、40％或更多)。在一些实施方案中，抗头痛药物选自5-HT1激动剂、曲普坦类、阿片类、β-肾上腺素能拮抗剂、麦角生物碱类和非类固醇类抗炎药 (non-steroidal anti-inflammatory drug，NSAID)。在一些实施方案中，抗头痛药物是曲普坦类。在一些实施方案中，施用于患者的单克隆抗体的量为约225mg至约1000mg，例如约675mg或约900mg。在一些实施方案中，单克隆抗体每月施用。在一些实施方案中，单克隆抗体作为单次剂量施用。在一些实施方案中，所述施用是皮下施用或静脉内施用。在一些实施方案中，单克隆抗体以至少150mg/mL的浓度配制。在一些实施方案中，其中单克隆抗体以小于2mL(例如约1.5mL)的体积施用。在一些实施方案中，对象是人。在一些实施方案中，单克隆抗体是人的或人源化的。在一些实施方案中，单克隆抗体包含：(a)具有SEQ ID NO:3所示CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO: 6所示CDR L1、SEQ ID NO:7所示CDR L2和SEQ ID NO:8所示CDR L3 的抗体；或(b)如表6中所示的根据(a)的抗体的变体。

[0019] 在一个方面，本发明提供了在对象中预防、治疗(持续性)创伤后头痛或降低其发病率的方法，其包括以调节CGRP途径的量向对象施用单次剂量的单克隆抗体(例如，单克隆抗CGRP拮抗剂抗体)，其中单克隆抗体的量为约225mg至约1000mg，例如约675mg或约900mg。

[0020] 在另一个实施方案中，本发明提供了用于在个体中预防、治疗、改善、控制(持续性)创伤后头痛、降低其发病率、或者延迟其发生或进展的方法，其包括向所述个体施用有效量的与至少一种可用于治疗创伤后头痛的另外的药剂组合的抗CGRP拮抗剂抗体。这样的另外的药剂包括5-HT1 样激动剂(和作用于其他5-HT1位点的激动剂)和非甾体抗炎药(NSAID)。

[0021] 可与抗CGRP抗体组合使用的5-HT1激动剂的实例包括称为曲普坦类的一类化合物，例如舒马普坦、佐米曲普坦、那拉曲普坦、利扎曲普坦、依来曲普坦、阿莫曲普坦和夫罗曲普坦。还已知麦角生物碱类及相关化合物具有5-HT激动剂活性。这些化合物包括酒石酸麦角胺、马来酸麦角新碱和甲磺酸二氢麦角碱(例如，双氢麦角考宁(dihydroergocornine)、双氢麦角汀(dihydroergocristine)、双氢麦角隐亭(dihydroergocryptine)和甲磺酸双氢麦角胺(DHE 45))。

[0022] 可与抗CGRP抗体组合使用的NSAID的实例包括阿司匹林、双氯芬酸、二氟尼柳(diflusinal)、依托度酸、芬布芬、非诺洛芬、氟苯柳(flufenisal)、氟比洛芬、布洛芬、吲哚美辛、酮洛芬、酮咯酸、甲氯芬那酸、甲芬那酸、萘丁美酮、萘普生、奥沙普秦(oxaprozin)、保泰松、吡罗昔康、舒林酸、托美汀或佐美酸、环氧合酶-2(COX-2)抑制剂(塞来昔布、罗非昔布、美洛昔康、JTE-522、L-745，337、NS398)、或其可药用盐。

[0023] 在一个实施方案中，用于上述任何方法的抗CGRP拮抗剂抗体是本文中所述的任何抗体。

[0024] 在一些实施方案中，抗CGRP拮抗剂抗体识别人CGRP。在一些实施方案中，抗CGRP拮抗剂抗体与人α-CGRP和β-CGRP都结合。在一些实施方案中，抗CGRP拮抗剂抗体与人和大鼠CGRP结合。在一些实施方案中，抗CGRP拮抗剂抗体与具有CGRP的第25至37位氨基酸的C端片段结合。在一些实施方案中，抗CGRP拮抗剂抗体与在CGRP的第25至 37位氨基酸内的C端表位结合。

[0025] 在一些实施方案中，抗CGRP拮抗剂抗体是单克隆抗体。在一些实施方案中，抗CGRP拮抗剂抗体是人源化的。在一些实施方案中，抗体是人的。在一些实施方案中，抗CGRP拮抗剂抗体是抗体G1(如本文中所述)。在一些实施方案中，抗CGRP拮抗剂抗体包含抗体G1或如表6中所示 G1变体的一个或更多个CDR(例如一个、两个、三个、四个、五个、或在一些实施方案中，全部六个CDR)。在另一些实施方案中，抗CGRP拮抗剂抗体包含图5中所示的重链可变区的氨基酸序列(SEQ ID NO:1)和图5中所示的轻链可变区的氨基酸序列(SEQ ID NO:2)。

[0026] 在一些实施方案中，抗体包含经修饰的恒定区，例如免疫惰性的(包括部分地免疫惰性的)恒定区，例如不触发补体介导的裂解、不刺激抗体依赖性细胞介导的细胞毒性(antibody-dependent cell mediated cytotoxicity, ADCC)、不激活小胶质细胞(microglia)、或具有降低的这些活性中的一种或更多种。在一些实施方案中，恒定区如Eur.J.Immunol.(1999) 29:2613-2624;PCT申请No.PCT/GB99/01441;和/或英国专利申请No. 9809951.8中所述进行修饰。在另一些实施方案中，抗体包含含有以下突变的人重链IgG2恒定区:A330P331至S330S331(参考野生型IgG2序列的氨基酸编号)。Eur.J.Immunol.(1999)29:2613-2624。在一些实施方案中，抗体的重链恒定区是具有以下任何突变的人重链IgG1:1)A327A330P331 至G327S330S331;2)E233L234L235G236(SEQ ID NO:48)至缺失G236 的P233V234A235;3)E233L234L235至P233V234A235;4)E233L234L235G236A327A330P331(SEQ ID NO:49)至缺失G236的P233V234A235G327S330S331(SEQ ID NO:50);5) E233L234L235A327A330P331(SEQ ID NO:51)至 P233V234A235G327S330S331(SEQ ID NO:50);以及6)N297至A297或除N之外的任何其他氨基酸。在一些实施方案中，抗体的重链恒定区是具有以下任何突变的人重链IgG4:E233F234L235G236(SEQ ID NO:52)至缺失G236的P233V234A235;E233F234L235至P233V234A235;以及 S228L235至P228E235。

[0027] 在另一些实施方案中，恒定区的N-连接的糖基化为非糖基化的。在一些实施方案中，通过使寡糖附接残基(例如Asn297)和/或作为恒定区中N-糖基化识别序列的一部分的侧翼残基突变来使恒定区的N连接的糖基化为非糖基化。在一些实施方案中，恒定区的N-连接的糖基化为非糖基化的。可以以酶促地或通过在糖基化缺陷型宿主细胞中表达来使恒定区的N-连接的糖基化为非糖基化。

[0028] 抗CGRP拮抗剂抗体与CGRP的结合亲和力(K_D)(例如人α-CGRP，如在合适温度如25℃或37℃下通过表面等离子体共振测量的)可以是约 0.02至约200nM。在一些实施方案中，结合亲和力为以下中的任一个:约200nM、约100nM、约50nM、约10nM、约1nM、约500pM、约100pM、约60pM、约50pM、约20pM、约15pM、约10pM、约5pM 或约2pM。在一些实施方案中，结合亲和力小于以下中的任一个:约250 nM、约200nM、约100nM、约50nM、约10nM、约1nM、约500pM、约100pM或约50pM。在一些实施方案中，结合亲和力小于约50nM。

[0029] 可在创伤后头痛之前、期间和/或之后施用抗CGRP拮抗剂抗体。在一些实施方案

中，在创伤后头痛发作之前(例如在头部和/或颈部受到创伤或损伤之后)施用抗CGRP拮抗剂抗体。抗CGRP拮抗剂抗体的施用可通过本领域中已知的任何方式来进行，包括：经口、静脉内、皮下、动脉内、肌内、鼻内(例如，具有或不具有吸入)、心内、脊柱内、胸内、腹膜内、室内、舌下、经皮、和/或通过吸入。施用可以是全身性的，例如静脉内，或局部的。在一些实施方案中，初始剂量和一个或更多个另外的剂量以相同的方式施用，即皮下或静脉内施用。在一些实施方案中，一个或更多个另外的剂量以与初始剂量不同的方式施用，即初始剂量可静脉内施用，而一个或更多个另外的剂量可皮下施用。

[0030] 在一些实施方案中，抗CGRP拮抗剂抗体可与另外的药剂(例如用于治疗创伤后头痛的另外的药剂)联合施用。

[0031] 在另一方面，本发明提供了抗CGRP拮抗剂抗体用于制备用于本文中所述任何方法(例如用于预防、治疗或减轻(持续性)创伤后头痛)的药物的用途。

[0032] 在另一方面，本发明提供了用于预防、治疗或减轻创伤后头痛的药物组合物，其包含有效量与一种或更多种可药用赋形剂组合的抗CGRP拮抗剂抗体。

[0033] 在另一方面，本发明提供了用于本文中所述任何方法的药盒(kit)。在一些实施方案中，药盒包含：容器、含有与可药用载体组合的本文中所述抗CGRP拮抗剂抗体的组合物、以及将组合物用于本文中所述任何方法的说明书。

[0034] 本发明还提供了来自抗体G1或其在表6中所示变体的抗CGRP拮抗剂抗体和多肽。因此，在一个方面，本发明提供了由具有ATCC登录号 PTA-6866和PTA-6867的表达载体产生的抗体G1(可互换地称为“G1”和“TEV-48125”)。例如，在一个实施方案中，抗体包含由具有ATCC登录号 PTA-6867的表达载体产生的重链。在另一个实施方案中，抗体包含由具有ATCC登录号PTA-6866的表达载体产生的轻链。图5中示出了G1的重链和轻链可变区的氨基酸序列。图5中还示出了抗体G1的互补决定区 (complementarity determining region,CDR)部分(包括Chothia和Kabat CDR)。应理解，对G1的任何部分或整个区域的提及涵盖由具有ATCC 登录号PTA-6866和PTA-6867的表达载体产生的序列和/或在图5中所描述的序列。在一些实施方案中，本发明还提供了具有表6中所描述的氨基酸序列的G1的抗体变体。

[0035] 在一个方面，本发明提供了包含V_H结构域的抗体，所述V_H结构域与SEQ ID NO:1在氨基酸序列方面具有至少85％、至少86％、至少87％、至少88％、至少89％、至少90％、至少91％、至少92％、至少93％、至少94％、至少95％、至少96％、至少97％、至少98％、至少99％或100％的同一性。

[0036] 在另一方面，本发明提供了包含V_L结构域的抗体，所述V_L结构域与 SEQ ID NO:2在氨基酸序列方面具有至少85％、至少86％、至少87％、至少88％、至少89％、至少90％、至少91％、至少92％、至少93％、至少94％、至少95％、至少96％、至少97％、至少98％、至少99％或100％的同一性。

[0037] 在另一方面，本发明提供了包含抗体G1或其在表6中所示变体的片段或区域的抗体。在一个实施方案中，所述片段是抗体G1的轻链。在另一个实施方案中，所述片段是抗体G1的重链。在另一个实施方案中，所述片段包含来自抗体G1的轻链和/或重链的一个或更多个可变区。在另一个实施方案中，所述片段包含图5中所示的来自轻链和/或重链的一个或更多个可变区。在另一个实施方案中，所述片段包含来自抗体G1的轻链和/或重链的一个或更多个CDR。

[0038] 在另一方面，本发明提供了包含SEQ ID NO:5所示V_H CDR3或与 SEQ ID NO:5的区别之处在于1、2、3、4或5个氨基酸替换的序列的多肽(其可以是或可以不是抗体)。在一个特别的实施方案中，这样的氨基酸替换是保守替换。

[0039] 在另一方面，本发明提供了包含SEQ ID NO:8所示V_L CDR3或与 SEQ ID NO:8的区别之处在于1、2、3、4或5个氨基酸替换的序列的多肽(其可以是或可以不是抗体)。在一个特别的实施方案中，这样的氨基酸替换是保守替换。

[0040] 在另一方面，本发明提供了包含以下任意一个或更多个的多肽(其可以是或可以不是抗体)：a)抗体G1或其在表6中所示变体的一个或更多个CDR；b)来自抗体G1或其在表6中所示变体的重链的CDR H3；c) 来自抗体G1或其在表6中所示变体的轻链的CDR L3；d)来自抗体G1 或其在表6中所示变体的轻链的三个CDR；e)来自抗体G1或其在表6 中所示变体的重链的三个CDR；f)抗体G1或其在表6中所示变体的来自轻链的三个CDR和来自重链的三个CDR。在一些实施方案中，本发明还提供了包含以下任意一个或更多个的多肽(其可以是或可以不是抗体)： a)来自抗体G1或其在表6中所示变体的一个或更多个(1、2、3、4、5 或6个)CDR；b)来自抗体G1的重链的CDR H3的CDR；和/或c)来自抗体G1的轻链的CDR L3的CDR。在一些实施方案中，CDR是图5 中所示的CDR。在一些实施方案中，来自抗体G1或其在表6中所示变体的一个或更多个CDR与G1或其变体的至少1个、至少2个、至少3个、至少4个、至少5个或至少6个CDR具有至少约85％、至少约86％、至少约87％、至少约88％、至少约89％、至少约90％、至少约91％、至少约92％、至少约93％、至少约94％、至少约95％、至少约96％、至少约 97％、至少约98％或至少约99％的同一性。

[0041] 在一些实施方案中，CDR是Kabat CDR。在另一些实施方案中，CDR 是Chothia CDR。在另一些实施方案中，CDR是Kabat CDR和Chothia CDR 的组合(也称为“组合CDR”或“扩展CDR”)。换言之，对于包含多于一个 CDR的任何给定实施方案，CDR可以是Kabat、Chothia和/或组合中的任一个。

[0042] 在一些实施方案中，多肽(例如抗体)包含KASKXaaVXaaTYVS(SEQ ID NO:53)的氨基酸序列，其中第5位的Xaa是R、W、G、L或N；并且其中第7位的Xaa是T、A、D、G、R、S、W或V。在一些实施方案中，KASKXaaVXaaTYVS(SEQ ID NO:53)的氨基酸序列是抗体轻链的CDR1。

[0043] 在一些实施方案中，多肽(例如抗体)包含XaaXaaSNRYXaa(SEQ ID NO: 54)的氨基酸序列，其中第1位的Xaa是G或A；其中第2位的Xaa是 A或H；并且其中第7位的Xaa是L、T、I或S。在一些实施方案中， XaaXaaSNRYXaa(SEQ ID NO:54)的氨基酸序列是抗体轻链的CDR2。

[0044] 在一些实施方案中，多肽(例如抗体)包含 EIRSXaaSDXaaXaaATXaaYAXaaAVKG(SEQ ID NO:55)的氨基酸序列，其中第5位的Xaa是E、R、K、Q或N；其中第8位的Xaa是A、G、N、E、 H、S、L、R、C、F、Y、V、D或P；其中第9位的Xaa是S、G、T、Y、 C、E、L、A、P、I、N、R、V、D或M；其中第12位的Xaa是H或F；其中第15位的Xaa是E或D。在一些实施方案中，EIRSXaaSDXaaXaaATXaaYAXaaAVKG(SEQ ID NO:55)的氨基酸序列是抗体重链的CDR2。

[0045] 在一些实施方案中，多肽(例如抗体)包含SEQ ID NO:1的氨基酸序列，其中SEQ ID NO:1的第99位的氨基酸残基是L或被A、N、S、T、 V或R替换；并且其中SEQ ID NO:1的第100位的氨基酸残基是A或被 L、R、S、V、Y、C、G、T、K或P替换。

[0046] 在一些实施方案中，抗体是人抗体。在另一些实施方案中，抗体是人源化抗体。在一些实施方案中，抗体是单克隆的。在一些实施方案中，抗体(或多肽)是分离的。在一些实

施方案中,抗体(或多肽)基本上是纯的。

[0047] 抗体的重链恒定区可以来自任何类型的恒定区,例如IgG、IgM、IgD、IgA和IgE;以及任何同种型,例如IgG1、IgG2、IgG3和IgG4。

[0048] 在一些实施方案中,抗体包含如本文中所述的经修饰恒定区。

[0049] 在另一方面,本发明提供了多核苷酸(其可以是分离的),其包含编码抗体G1或其在表6中所示变体的片段或区域的多核苷酸。在一个实施方案中,所述片段是抗体G1的轻链。在另一个实施方案中,所述片段是抗体G1的重链。在另一个实施方案中,所述片段包含来自抗体G1的轻链和/或重链的一个或更多个可变区。在另一个实施方案中,所述片段包含来自抗体G1的轻链和/或重链的一个或更多个(即,1、2、3、4、5或 6个)互补决定区(CDR)。

[0050] 在另一方面,本发明提供了多核苷酸(其可以是分离的),其包含编码抗体G1或其在表6中所示变体的多核苷酸。在一些实施方案中,多核苷酸包含SEQ ID NO:9和SEQ ID NO:10中所示的多核苷酸中的任一个或二者。

[0051] 在另一方面,本发明提供了编码本文中所述的任何抗体(包括抗体片段)或多肽的多核苷酸。

[0052] 在另一方面,本发明提供了包含本文中公开的任何多核苷酸的载体 (包括表达载体和克隆载体)和宿主细胞。在一些实施方案中,载体是具有ATCC No.PTA-6867的pDb.CGRP.hFcGI。在另一些实施方案中,载体是具有ATCC No.PTA-6866的pEb.CGRP.hKGI。

[0053] 在另一方面,本发明提供了包含编码本文中所述的任何抗体的多核苷酸的宿主细胞。

[0054] 在另一方面,本发明提供了被本文中所述的任何抗体或多肽结合的 CGRP的复合物。在一些实施方案中,抗体是抗体G1或其在表6中所示变体。

[0055] 在另一方面,本发明提供了药物组合物,其包含有效量的本文中所述的任何多肽(包括抗体,例如包含抗体G1的一个或更多个CDR的抗体) 或多核苷酸,以及可药用赋形剂。

[0056] 在另一方面,本发明提供了产生抗体G1的方法,其包括:在允许产生抗体G1的条件下培养宿主细胞或其后代,其中所述宿主细胞包含编码抗体G1的表达载体;以及在一些实施方案中,纯化抗体G1。在一些实施方案中,表达载体包含SEQ ID NO:9和SEQ ID NO:10中所示的多核苷酸序列中的任一个或二者。

[0057] 在另一方面,本发明提供了如下产生本文中所述的任何抗体或多肽的方法:在合适的细胞中表达编码抗体(其可作为单一轻链或重链单独表达,或者由一个载体表达轻链和重链二者)或多肽的一种或更多种多核苷酸,通常随后回收和/或分离目的抗体或多肽。

[0058] 本发明的抗CGRP拮抗剂抗体和多肽以及编码抗体和多肽的多核苷酸可用于预防、治疗、防止、改善、控制与CGRP异常功能相关的疾病或降低其发病率,所述疾病例如创伤后头痛和可通过拮抗CGRP活性而预防、治疗或防止的其他病症。

[0059] 在另一方面,本发明提供了包含本文中所述的任意一种或更多种组合物的药盒和组合物。通常在合适的包装中并提供有合适的说明书的这些药盒可用于本文中所述的任何方法。

[0060] 在一个方面,本发明提供了根据本文中所述的任何方法使用的组合物。

[0061] 在一个方面,本发明提供了用于降低对象经历的每月头痛小时数的组合物。在一个实施方案中,使用包括向对象施用一定量的调节CGRP途径的单克隆抗体,其中所述单克

隆抗体的量在单次剂量之后有效地使每月头痛小时数降低至少20(例如,25、30、35、40、45、50、55、60、65、 70个或更多个头痛小时)。在一些实施方案中,每月头痛小时数降低至少约50个小时。在一个实施方案中,使用包括向对象施用一定量的调节 CGRP途径的单克隆抗体,其中所述单克隆抗体的量在单次剂量之后有效地使每月头痛小时数降低至少15%(例如20%、25%、30%、35%、40%或更多)。在一些实施方案中,每月头痛小时数降低至少约30%。在一些实施方案中,单克隆抗体是抗CGRP拮抗剂抗体。在一些实施方案中,施用于患者的单克隆抗体的量为约675mg至约1000mg。在一些实施方案中,单克隆抗体每月施用。在一些实施方案中,单克隆抗体作为单次剂量施用。在一些实施方案中,所述施用是皮下施用或静脉内施用。在一些实施方案中,单克隆抗体以至少150mg/mL的浓度配制。在一些实施方案中,其中单克隆抗体以小于2mL的体积施用。在一些实施方案中,对象是人。在一些实施方案中,单克隆抗体是人的或人源化的。在一些实施方案中,单克隆抗体包含:(a)具有SEQ ID NO:3所示CDR H1、SEQ ID NO:4 所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6所示CDR L1、 SEQ ID NO:7所示CDR L2和SEQ ID NO:8所示CDR L3的抗体;或(b) 如表6中所示的根据(a)的抗体的变体。

[0062] 在一个方面,本发明提供了用于降低对象经历的每月头痛日数的组合物。在一个实施方案中,使用包括向对象施用一定量的调节CGRP途径的单克隆抗体,其中所述单克隆抗体的量在单次剂量之后有效地使每月头痛日数降低至少3(例如3、4、5、6、7、8、9、10、11、12、13、14、15、 16、17、18、19、20或更多个头痛日)。在一些实施方案中,每月头痛日数降低至少约6个头痛日。在一些实施方案中,单克隆抗体是抗CGRP拮抗剂抗体。在一些实施方案中,施用于患者的单克隆抗体的量为约675mg 至约1000mg。在一些实施方案中,单克隆抗体每月施用。在一些实施方案中,单克隆抗体作为单次剂量施用。在一些实施方案中,所述施用是皮下施用或静脉内施用。在一些实施方案中,单克隆抗体以至少150mg/mL 的浓度配制。在一些实施方案中,其中单克隆抗体以小于2mL(例如约 1.5mL)的体积施用。在一些实施方案中,对象是人。在一些实施方案中,单克隆抗体是人的或人源化的。在一些实施方案中,单克隆抗体包含:(a) 具有SEQ ID NO:3所示CDR H1、SEQ ID NO:4所示CDR H2、SEQ ID NO: 5所示CDR H3、SEQ ID NO:6所示CDR L1、SEQ ID NO:7所示CDR L2 和SEQ ID NO:8所示CDR L3的抗体;或(b)如表6中所示的根据(a) 的抗体的变体。

[0063] 在一个方面,本发明提供了用于降低对象中任何急性头痛药物的使用的组合物,其包括向对象施用调节CGRP途径的单克隆抗体(例如,抗 CGRP拮抗剂抗体),其中所述单克隆抗体的量有效地使对象对急性头痛药物的每月使用降低至少15%(例如20%、25%、30%、35%、40%或更多)。在一些实施方案中,抗头痛药物选自:5-HT1激动剂、曲普坦类、阿片类、β-肾上腺素能拮抗剂、麦角生物碱类和非甾体抗炎药(NSAID)。在一些实施方案中,抗头痛药物是曲普坦类。在一些实施方案中,施用于患者的单克降抗体的量为约675mg至约1000mg。在一些实施方案中,单克隆抗体每月施用。在一些实施方案中,单克隆抗体作为单次剂量施用。在一些实施方案中,所述施用是皮下施用或静脉内施用。在一些实施方案中,单克隆抗体以至少150mg/mL的浓度配制。在一些实施方案中,其中单克隆抗体以小于2mL(例如约1.5mL)的体积施用。在一些实施方案中,对象是人。在一些实施方案中,单克隆抗体是人的或人源化的。在一些实施方案中,单克隆抗体包含:(a)具有SEQ ID NO:3所示CDR H1、 SEQ ID NO:4所示CDR H2、SEQ ID NO:5所示CDR H3、SEQ ID NO:6 所示CDR L1、SEQ ID

NO:7所示CDR L2和SEQ ID NO:8所示CDR L3 的抗体;或(b)如表6中所示的根据(a)的抗体的变体。

[0064] 在一个方面,本发明提供了组合物,其用于在对象中预防、治疗创伤后头痛或降低其发病率,包括以调节CGRP途径的量向对象施用单次剂量的单克隆抗体(例如,单克隆抗CGRP拮抗剂抗体),其中施用于患者的单克隆抗体的量为约675mg至约1000mg。

[0065] 附图简述

[0066] 图1是示出了12种鼠抗体对不同的经丙氨酸替换经人α-CGRP片段的结合亲和力的表格。结合亲和力通过使Fab流过芯片上的CGRP使用Biacore在25℃下测量。加框的值表示丙氨酸突变体相对于亲本片段25-37 (斜体)的亲和力损失,除来自于19-37亲本的K35A之外。"[a]"表示对19-37 和25-37片段的亲和力是在不同传感器芯片上的两次独立测量的平均值±标准差。"[b]"表示这些相互作用由于双相解离率而偏离简单的双分子相互作用模型,因此其亲和力使用构象变化模型来确定。灰度色调:白色(1.0) 表示亲本亲和力;浅灰(小于0.5)表示比亲本更高的亲和力;暗灰(大于2)表示比亲本更低的亲和力;黑色表示未检测到结合。

[0067] 图2A和2B示出了在电脉冲刺激30秒之后作为血细胞通量测量的施用CGRP 8-37 (400nmol/kg)、抗体4901(25mg/kg)和抗体7D11(25mg/kg) 对皮肤血流的作用。CGRP 8-37在电脉冲刺激之前3至5分钟静脉内(iv) 施用。抗体在电脉冲刺激之前72小时腹膜内(IP)施用。图中的每个点表示在所示条件下处理的一只大鼠的AUC。图中的每条线代表在所示条件下处理的大鼠的平均AUC。AUC(曲线下面积)等于Δ通量xΔ时间。"Δ通量"表示电脉冲刺激之后的通量单位的变化;并且"Δ时间"表示血细胞通量水平恢复至电脉冲刺激之前水平所需的时间。

[0068] 图3示出了在电脉冲刺激30秒之后作为血细胞通量测量的施用不同剂量的抗体4901(25mg/kg、5mg/kg、2.5mg/kg或1mg/kg)对皮肤血流的作用。抗体在电脉冲刺激之前24小时静脉内(IV)施用。图中的每个点表示在所示条件下处理的一只大鼠的AUC。图中的线代表在所示条件下处理的大鼠的平均AUC。

[0069] 图4A和4B示出了在电脉冲刺激30秒之后作为血细胞通量测量的施用抗体4901(1mg/kg或10mg/kg,i.v.)、抗体7E9(10mg/kg,i.v.)和抗体8B6(10mg/kg,i.v.)对皮肤血流的作用。静脉内(i.v.)施用抗体,随后在抗体施用之后30分钟、60分钟、90分钟和120分钟进行电脉冲刺激。Y轴代表与未施用抗体时(时间0)的AUC水平相比的AUC百分比。 X轴代表在施用抗体与电脉冲刺激之间的时间(分钟)。"*"表示与时间0 相比P＜0.05,"**"表示与时间0相比P＜0.01。数据使用具有Dunnett多重比较检验的单因素ANOVA进行分析。

[0070] 图5示出了抗体G1的重链可变区(SEQ ID NO:1)和轻链可变区(SEQ ID NO:2)的氨基酸序列。Kabat CDR为粗体文本,且Chothia CDR加有下划线。重链可变区和轻链可变区的氨基酸残基按顺序编号。

[0071] 图6示出了使用Biacore通过肽竞争对抗体G1的表位作图。将N-生物素化的人α-CGRP捕获在SA传感器芯片上。使不存在竞争性肽或与10 μM竞争性肽预孵育1小时的G1 Fab(50nM)流动到芯片上。测量G1 Fab 与芯片上人α-CGRP的结合。Y轴代表与不存在竞争性肽下的结合相比由于竞争性肽的存在而阻断的结合的百分比。

[0072] 图7示出了在电脉冲刺激30秒之后作为血细胞通量测量的施用抗体 G1(1mg/kg或

10mg/kg,i.v.)或载剂(PBS,0.01％Tween 20)对皮肤血流的作用。静脉内(i.v.)施用抗体G1或载剂,随后在抗体施用之后30 分钟、60分钟、90分钟和120分钟进行神经电脉冲刺激。Y轴代表与未施用抗体或载剂时(时间0)的AUC水平(限定为100％)相比的AUC 百分比。X轴代表在施用抗体与电脉冲刺激之间的时间(分钟)。“*”表示与载剂相比P＜0.05,“**”表示与载剂相比P＜0.01。数据使用双因素 ANOVA和Bonferroni事后检验进行分析。

[0073] 图8A示出了给药之后24小时在电脉冲刺激30秒之后作为血细胞通量测量的施用抗体G1(1mg/kg、3mg/kg或10mg/kg,iv)或载剂(PBS， 0.01％Tween 20)对皮肤血流的作用。抗体G1或载剂在神经电脉冲刺激之前24小时静脉内(i.v.)施用。Y轴代表总曲线下面积(从刺激开始到通量恢复至基线的血细胞通量变化乘以时间变化,AUC)。X轴代表抗体 G1的变化剂量。“*”表示与载剂相比P＜0.05,“**”表示与载剂相比P＜0.01。数据使用单因素ANOVA和Dunn多重比较检验进行分析。

[0074] 图8B示出了给药之后7天在电脉冲刺激30秒之后作为血细胞通量测量的施用抗体G1(0.3mg/kg、1mg/kg、3mg/kg或10mg/kg,i.v.)或载剂 (PBS,0.01％Tween 20)对皮肤血流的作用。抗体G1或载剂在神经电脉冲刺激之前7天静脉内(i.v.)施用。Y轴代表总AUC。X轴代表抗体G1 的变化剂量。“**”表示与载剂相比P＜0.01,“***”表示与载剂相比P＜0.001。数据使用单因素ANOVA和Dunn多重比较检验进行分析。

[0075] 图8C是来自图8A和8B的数据的曲线拟合分析。抗体G1或载剂在神经电脉冲刺激之前24小时或7天静脉内(i.v.)施用。Y轴代表总AUC。 X轴代表确定EC_{50}而在对数标度上的以“mg/kg”计的抗体G1的变化剂量。

[0076] 图9示出了在电场刺激之后抗体mu7E9(10mg/kg)、BIBN4096BS 或载剂(PBS，0.01％Tween 20)对脑膜中动脉的直径变化的作用。抗体 mu7E9、BIBN4096BS或载剂在建立针对电刺激的基线响应之后在时间点 0分钟静脉内(i.v.)施用。Y轴代表电场刺激之后脑膜中动脉的直径变化。静止直径对应于0％。X轴代表电脉冲刺激的时间(分钟)。“*”表示与载剂相比P＜0.05,“**”表示与载剂相比P＜0.01。数据使用单因素ANOVA 和Dunett多重比较检验进行分析。

[0077] 图10示出了在电场刺激之后不同剂量的抗体G1(1mg/kg、3mg/kg 或10mg/kg，i.v.)或载剂(PBS,0.01％Tween 20)对脑膜中动脉的直径变化的作用。抗体G1或载剂在电场刺激之前7天静脉内(i.v.)施用。Y 轴代表脑膜中动脉的直径变化。静止直径对应于0％。X轴代表刺激电压。“*”表示与载剂相比P＜0.05,“**”表示与载剂相比P＜0.01,并且″***″表示与载剂相比P＜0.001。数据使用双因素ANOVA和Bonferroni事后检验进行分析。

[0078] 图11A示出了24小时前静脉内(i.v.)施用的抗体mu4901(10mg/kg) 或载剂(PBS，0.01％Tween 20)对吗啡成瘾大鼠中由皮下注射纳洛酮(1 mg/kg)诱导的体核温度降低的作用。Y轴代表与基线的温差。X轴代表从纳洛酮注射点开始测量的时间。

[0079] 图11B 示出了24小时前静脉内(i.v.)施用的抗体mu4901(10mg/kg) 或载剂(PBS，0.01％Tween 20)对吗啡成瘾大鼠中由皮下注射纳洛酮(1 mg/kg)诱导的尾表面温度升高的作用。Y轴代表与基线的温差。X轴代表从纳洛酮注射点开始测量的时间。

[0080] 图12A和12B是显示mCHI对(12A)垂直探究活动和(12B)新物体识别的作用的两个条形图。数据是平均值+SEM(n＝8);*p＜0.05,相对于假手术。

[0081] 图13A至13D是显示在mCHI之后的14天中针对机械刺激的头部(13A和13C)和后爪

(13B和13D)响应阈值和累积伤害感受评分的四个线图。数据是平均值+SEM(n＝6)。*p＜0.05,相对于假手术。

[0082] 图14A至14D是显示mCHI之后急性舒马普坦(图14A和14B)或长期鼠抗CGRP抗体(图14C和14D)处理对针对机械刺激的响应阈值和累积伤害感受评分的作用的四个线图。数据是平均值+SEM(n＝6-8)。*p＜0.05,假手术相对于mCHI;#p＜0.05,mCHI相对于mCHI+舒马普坦 (Suma);+p＜0.05,对照IgG相对于抗CGRP mAb。

[0083] 图15A和15B示出了在条件化日(conditioning day)期间用于条件化位置偏好的处理方案(图15A),并且显示舒马普坦在mCHI之后7天在动物中产生CPP,而在假手术动物中则没有(图15B)。数据是平均值+SEM (n＝8)。**p＜0.001盐水配对相对于舒马普坦配对。

[0084] 图16A至16D是显示mCHI之后第15天和第30天的GTN施用对头部机械超敏性的作用的四个条形图。数据是平均值+SEM(n＝8);*p＜0.05, **p＜0.01,***p＜0.001,相对于假手术。

[0085] 图17A至17D是显示mCHI之后第15天和第30天的GTN施用对后爪机械超敏性的作用的四个条形图。数据是平均值+SEM(n＝8);*p＜0.05,相对于假手术。

[0086] 图18A至18D是显示与对应GTN前第14天值相比GTN之后4小时急性舒马普坦处理或长期抗CGRP mAb施用及其对照处理对％响应阈值降低(图18A和18C)或％伤害感受评分提高(图18B和18D)的作用的四个条形图。数据是平均值+SEM(n＝8);*p＜0.05,**p＜0.01,***p＜0.001,相对于GTN+载剂(Veh)或GTN+IgG。

[0087] 图19A和19B示出了在条件化日期间用于条件化位置厌恶的处理方案(图19A),并且显示GTN在注射了IgG的mCHI动物中在受损之后第 14天产生位置性厌恶,而在用抗CGRP mAb处理的mCHI动物中则没有 (图19B)。数据是平均值+SEM(n＝8)。*p＜0.05,GTN前配对室相对于 GTN配对室。

[0088] 发明详述

[0089] 在一些方面,本文中公开的发明提供了通过向个体施用治疗有效量的抗CGRP拮抗剂抗体来在所述个体中预防、治疗和/或减轻创伤后头痛的方法。

[0090] 在一些方面,本文中公开的发明还提供了来自G1或其在表6中所示变体的抗CGRP拮抗剂抗体和多肽。在一些实施方案中,本发明还提供了制备和使用这些抗体和多肽的方法。

[0091] 一般性技术

[0092] 除非另有指明,否则本发明的各个方面的实践将采用在本技术领域范围内的分子生物学(包括重组技术)、微生物学、细胞生物学、生物化学和免疫学的常规技术。这样的技术在例如以下的文献中有充分说明: Molecular Cloning:A Laboratory Manual,第二版(Sambrook等,1989)Cold Spring Harbor Press;Oligonucleotide Synthesis(M.J.Gait,编辑,1984); Methods in Molecular Biology,Humana Press;Cell Biology:A Laboratory Notebook(J.E.Cellis,编辑,1998)Academic Press;Animal Cell Culture(R.I. Freshney,编辑,1987);Introduction to Cell and Tissue Culture(J.P.Mather和P.E.Roberts,1998)Plenum Press;Cell and Tissue Culture:Laboratory Procedures(A.Doyle,J.B.Griffiths,和D.G.Newell,编辑,1993-1998)J.Wiley 和Sons;Methods in Enzymology(Academic Press,Inc.);Handbook of Experimental Immunology(D.M.Weir

和C.C.Blackwell,编辑);Gene Transfer Vectors for Mammalian Cells(J.M.Miller和M.P.Calos,编辑, 1987);Current Protocols in Molecular Biology(F.M.Ausubel等,编辑, 1987);PCR:The Polymerase Chain Reaction,(Mullis等,编辑,1994);Current Protocols in Immunology(J.E.Coligan等,编辑,1991);Short Protocols in Molecular Biology(Wiley和Sons,1999);Immunobiology(C.A.Janeway 和P.Travers,1997);Antibodies(P.Finch,1997);Antibodies:a practical approach(D.Catty.,编辑IRL Press,1988-1989);Monoclonal antibodies:a practical approach(P.Shepherd和C.Dean,编辑,Oxford University Press, 2000):Using antibodies:a laboratory manual(E.Harlow和D.Lane(Cold Spring Harbor Laboratory Press,1999));The Antibodies(M.Zanetti和J.D. Capra,编辑,Harwood Academic Publishers,1995)。

[0093] 定义

[0094] 本文使用的“约”当用于提及数值范围、截止值或特定值时用于指示所记载的值可与所列出的值相差最多多至10%。因此,术语“约”用于涵盖与指定值的±10%或更小的变化、±5%或更小的变化、±1%或更小的变化、±0.5%或更小的变化、或者±0.1%或更小的变化。

[0095] “抗体”是能够通过位于免疫球蛋白分子的可变区中的至少一个抗原识别位点与靶标(例如碳水化合物、多核苷酸、脂质、多肽等)特异性结合的免疫球蛋白分子。本文中使用的该术语不仅涵盖完整的多克隆抗体或单克隆抗体,而且还涵盖其片段(例如Fab、Fab′、$F(ab')_2$、Fv)、单链(ScFv)、其突变体、包含抗体部分(例如结构域抗体)的融合蛋白,以及包含抗原识别位点的免疫球蛋白分子的任何其他经修饰构型。抗体包括任何类别的抗体,例如IgG、IgA或IgM(或其亚类),并且抗体不需要是任何特定的类别。取决于其重链的恒定结构域的抗体氨基酸序列,可将免疫球蛋白分为不同的类别。有五种主要类别的免疫球蛋白:IgA、IgD、IgE、IgG和 IgM,并且这些中的数种可进一步分成亚类(同种型),例如IgG1、IgG2、IgG3、IgG4、IgA1和IgA2。对应于不同类别的免疫球蛋白的重链恒定结构域分别被称为α、δ、ε、γ和μ。不同类别的免疫球蛋白的亚基结构和三维构型是公知的。

[0096] 本文使用的“单克隆抗体”是指从基本上同质的抗体的群体获得的抗体,即除了可以以少量存在的可能的天然突变之外,构成该群体的单独抗体是相同的。单克隆抗体是高度特异性的,针对单一抗原位点。此外,与通常包含针对不同决定簇(表位)的不同抗体的多克隆抗体制备物相反,每种单克隆抗体针对抗原上的单一决定簇。修饰语“单克隆的”表示抗体从基本上同质的抗体群获得的特征,并且不被解释为需要通过任何特定方法产生抗体。例如,根据本发明使用的单克隆抗体可通过首先由Kohler和 Milstein,1975,Nature,256:495描述的杂交瘤方法制备,或者可通过例如美国专利No.4,816,567中所述的重组DNA方法制备。单克隆抗体还可以从使用例如McCafferty等,1990,Nature,348:552-554中所述的技术产生的噬菌体文库中分离。

[0097] 本文使用的“人源化”抗体是指作为以下非人(鼠)抗体形式:包含来自非人免疫球蛋白的最小序列的特异性嵌合免疫球蛋白、免疫球蛋白链或其片段(例如抗体的Fv、Fab、Fab′、$F(ab')_2$或其他抗原结合子序列)。在大多数情况下,人源化抗体是以下人免疫球蛋白(接受者抗体),其中来自接受者之互补决定区(CDR)的残基被来自非人物种之CDR的具有期望特异性、亲和力和生物活性的残基(供体抗体)替代,所述非人物种例如小鼠、大鼠或兔。

在一些情况下，人免疫球蛋白的Fv框架区(FR)残基被对应的非人残基替代。此外，人源化抗体可包含既不存在于接受者抗体中也不存在于导入的CDR或框架序列中的残基，但被包含在内以进一步改进和优化抗体性能。通常来说，人源化抗体将包含至少一个并且通常两个可变结构域的基本上所有，其中所有或基本上所有的CDR区对应于非人免疫球蛋白的那些，并且所有或基本上所有的FR区是人免疫球蛋白共有序列的那些。人源化抗体最佳地还将包含免疫球蛋白恒定区或结构域 (Fc)(通常是人免疫球蛋白的)的至少一部分。抗体可具有如WO 99/58572中所述修饰的Fc区。人源化抗体的另一些形式具有相对于原始抗体改变的一个或更多个(1、2、3、4、5、6个)CDR，其也被称为“来自于”原始抗体的一个或更多个CDR的一个或更多个CDR。

[0098] 本文使用的“人抗体”意指具有对应于由人产生的抗体的氨基酸序列之氨基酸序列的抗体和/或已经使用本领域中已知或本文中公开的任何用于制备人抗体的技术制备。人抗体的该定义包括包含至少一个人重链多肽或至少一个人轻链多肽的抗体。一个这样的实例是包含鼠轻链和人重链多肽的抗体。人抗体可使用本领域中已知的多种技术来产生。在一个实施方案中，人抗体从噬菌体文库中选择，其中该噬菌体文库表达人抗体 (Vaughan等，1996，Nature Biotechnology，14:309-314；Sheets等，1998， PNAS，(USA)95:6157-6162；Hoogenboom和Winter，1991，J.Mol.Biol.， 227:381；Marks等，1991，J.Mol.Biol.，222:581)。人抗体也可通过将人免疫球蛋白基因座引入转基因动物中来制备，所述转基因动物例如其中内源性免疫球蛋白基因已部分或完全失活的小鼠。这种方法在美国专利No. 5,545,807、5,545,806、5,569,825、5,625,126、5,633,425和5,661,016中描述。或者，人抗体可通过永生化产生针对靶抗原的抗体的人B淋巴细胞来制备(这样的B淋巴细胞可从个体中回收或者可在体外免疫接种)。参见例如Cole等，Monoclonal Antibodies and Cancer Therapy，Alan R.Liss，第77 页(1985)；Boemer等，1991，J.Immunol.，147(1):86-95；和美国专利No. 5,750,373。

[0099] 本文使用的术语“降钙素基因相关肽”和“CGRP”是指保留CGRP活性的至少一部分的任何形式的降钙素基因相关肽及其变体。例如，CGRP可以是α-CGRP或β-CGRP。本文使用的CGRP包括所有哺乳动物物种(例如人、犬、猫、马和牛)的天然序列CGRP。

[0100] 本文使用的“抗CGRP拮抗剂抗体”(可互换地称为“抗CGRP抗体”) 是指能够与CGRP结合并抑制CGRP生物活性和/或由CGRP信号传导介导的下游途径的抗体。抗CGRP拮抗剂抗体涵盖调节、阻断、拮抗、抑制或降低(包括显著地)CGRP生物活性或以其他方式拮抗CGRP途径的抗体，所述途径包括由CGRP信号传导介导的下游途径，例如受体结合和/ 或针对CGRP的细胞响应的激发。出于本发明的目的，将明确理解，术语“抗CGRP拮抗剂抗体”涵盖CGRP本身、CGRP生物活性(包括但不限于其介导任何头痛方面的能力)或生物活性的结果在任何有意义的程度上基本上消除、降低或中和的所有先前确定的术语、标题以及功能状态和特征。在一些实施方案中，抗CGRP拮抗剂抗体结合CGRP并阻止CGRP与 CGRP受体结合。在另一些实施方案中，抗CGRP抗体结合CGRP并阻止 CGRP受体的激活。本文中提供了抗CGRP拮抗剂抗体的一些实例。

[0101] 本文使用的术语“G1”、“抗体G1”和“TEV-48125”可互换使用，是指由具有保藏号ATCC PTA-6867和ATCC PTA 6866的表达载体产生的抗CGRP拮抗剂抗体。图5中示出了重链和轻链可变区的氨基酸序列。图5 中图示了抗体G1的CDR部分(包括Chothia和Kabat CDR)。

编码重链和轻链可变区的多核苷酸示于SEQ ID NO:9和SEQ ID NO:10。在实施例中描述了G1的表征。

[0102] 术语“多肽”、“寡肽”、“肽”和“蛋白质”在本文中可互换使用以指任何长度的氨基酸聚合物。聚合物可以是直链的或支链的,其可包含经修饰的氨基酸,并且其可被非氨基酸中断。这些术语还涵盖已被天然修饰或通过干预修饰的氨基酸聚合物;例如二硫键形成、糖基化、脂化、乙酰化、磷酸化或任何其他操作或修饰,例如与标记组分缀合。该定义中还包括例如包含一个或更多个氨基酸类似物(包括例如非天然氨基酸等)以及本领域中已知的其他修饰的多肽。应理解,由于本发明的多肽基于抗体,因此多肽可作为单链或缔合链(associated chain)存在。

[0103] 本文中可互换使用的“多核苷酸”或“核酸”是指任何长度的核苷酸聚合物,并且包括DNA和RNA。核苷酸可以是脱氧核糖核苷酸、核糖核苷酸、经修饰的核苷酸或碱基和/或其类似物,或可通过DNA或RNA聚合酶掺入聚合物中的任何底物。多核苷酸可包含经修饰的核苷酸,例如甲基化核苷酸及其类似物。如果存在的话,可在组装聚合物之前或之后赋予对核苷酸结构的修饰。核苷酸序列可被非核苷酸组分中断。多核苷酸可在聚合之后进一步修饰,例如通过与标记组分缀合。另一些类型的修饰包括例如“帽”;用类似物替换一个或更多个天然核苷酸;核苷酸间修饰,例如具有不带电荷的连接(例如,膦酸甲酯、磷酸三酯、氨基磷酸酯、氨基甲酸酯等)和具有带电荷的连接(例如,硫代磷酸酯、二硫代磷酸酯等)的那些、含有侧基部分(例如如蛋白质(例如,核酸酶、毒素、抗体、信号肽、 ply-L-赖氨酸等))的那些、具有嵌入剂(例如,吖啶、补骨脂素等)的那些、含有螯合剂(例如,金属、放射性金属、硼、氧化金属等)的那些、含有烷化剂的那些、具有经修饰的连接的那些(例如,α端基异构核酸(alpha anomeric nucleic acid)等),以及多核苷酸的未经修饰形式。此外,通常存在于糖中的任何羟基可例如被膦酸酯基团、磷酸酯基团替代,被标准保护基团保护、或被活化以制备与另外的核苷酸的另外的连接,或者可与固体支持物缀合。5′和3′端OH可被磷酸化或被1至20个碳原子的胺或有机封端基团部分(organic capping group moiety)取代。其他羟基也可被衍生化成标准保护基团。多核苷酸还可包含本领域中公知的核糖或脱氧核糖的类似形式,包括例如2′-O-甲基-、2′-O-烯丙基、2′-氟-或2′-叠氮基-核糖、碳环糖类似物、α-端基异构糖、差向异构糖(例如阿拉伯糖、木糖或来苏糖)、吡喃糖、呋喃糖、景天庚酮糖、无环类似物和无碱基核苷类似物(例如甲基核苷)。一个或更多个磷酸二酯连接可被作为替代的连接基团取代。这些作为替代的连接基团包括但不限于其中磷酸酯被P(O)S(“硫代酸酯”)、P(S)S(“二硫代酸酯”)、(O)NR_2(“酰胺化物”)、P(O)R、P(O)OR′、 CO或CH_2(“甲缩醛(formacetal)”)替换的一些实施方案,其中每个R 或R′独立地是H或者经取代或未经取代的烷基(1-20C),其任选地包含醚(-O-)连接、芳基、烯基、环烷基、环烯基或芳烃基(araldyl)。不是多核苷酸中的所有连接都需要相同。前面的描述适用于本文中提及的所有多核苷酸,包括RNA和DNA。

[0104] 本文使用的“创伤后头痛”是由头部和/或颈部的创伤或损伤引起的头痛,如The International Classification of Headache Disorders,第3版(β版), Cephalalgia,33(9):629-808(2013)中进一步所述。例如,创伤后头痛可类似于紧张型头痛或偏头痛。因此,其诊断在很大程度上取决于创伤或损伤与头痛发作之间的密切时间关系。与ICHD-II的诊断标准一致,ICHD-3β对所有亚型的诊断标准都要求必须报告在创伤或损伤7天内发生头

痛,或者在恢复意识和/或感知能力后7天内发生头痛并且在这些在创伤或损伤后已丧失时报告疼痛。虽然该7天间隔有些武断,并且虽然一些专家主张在少数患者中可在更长间隔后发生头痛,但目前没有足够的证据来改变该要求。

[0105] 熟练的从业者将能够容易地识别患有创伤后头痛的对象。例如,已发生头部的创伤性损伤,并且报告已在以下一种情况之后的7天内发生头痛:头部损伤、头部损伤后恢复意识、或停止损害头部损伤后感知或报告头痛之能力的药物。在一些情况下,在头部损伤之后3个月内头痛已经消退,或者头痛尚未消退,但自头部损伤起尚未超过3个月。

[0106] 可包括由创伤性头部损伤引起的少于3个月持续时间的头痛的用于由创伤性头部损伤引起的急性头痛的诊断标准可包括:

[0107] A.任何符合标准C和D的头痛

[0108] B.已发生创伤性头部损伤

[0109] C.报告已在以下一种情况之后的7天内发生头痛:

[0110] 1.头部损伤

[0111] 2.头部损伤后恢复意识

[0112] 3.停止头部损伤后损害感知或报告头痛之能力的药物

[0113] D.以下任一项:

[0114] 1.在头部损伤之后3个月内头痛已经消退

[0115] 2.头痛尚未消退,但自头部损伤起尚未超过3个月

[0116] E.通过其他ICHD-3诊断不能更好地解释。

[0117] 由中度或重度头部创伤性损伤引起的急性头痛的诊断标准可包括与以下至少一种相关的头部损伤:

[0118] 1.意识丧失＞30分钟

[0119] 2.格拉斯哥昏迷量表(GCS)评分＜13

[0120] 3.创伤后遗忘症1持续＞24小时

[0121] 4.意识水平改变＞24小时

[0122] 5.创伤性头部损伤的影像学证据,例如颅内出血和/或脑挫伤。

[0123] 由轻度创伤性头部损伤引起的创伤后头痛的诊断标准可包括满足以下这两项的头部损伤:

[0124] 1.与以下任一项都不相关:

[0125] a)意识丧失＞30分钟

[0126] b)格拉斯哥昏迷量表(GCS)评分＜13

[0127] c)创伤后遗忘症持续＞24小时

[0128] d)意识水平改变＞24小时

[0129] e)创伤性头部损伤的影像学证据,例如颅内出血和/或脑挫伤

[0130] 2.在头部损伤后立即与以下一种或更多种症状和/或体征相关:

[0131] a)暂时性意识错乱、定向障碍或意识受损

[0132] b)头部损伤之前或之后立即丧失记忆力

[0133] c)表明轻度创伤性脑损伤的两种或更多种其他症状:恶心、呕吐、视觉障碍、头晕和/或眩晕、记忆力和/或注意力受损。

[0134] 据报道,由创伤性头部损伤引起的持续性创伤后头痛是由头部创伤性损伤引起的持续时间超过三个月的头痛,并且报告已在以下一种情况之后的7天内发生头痛:头部损伤、头部损伤后恢复意识、或停止损害头部损伤后感知或报告头痛之能力的药物。在一些情况下,在头部损伤后,头痛持续超过三个月。

[0135] 由中度或重度创伤性头部损伤引起的持续性头痛的诊断标准可包括与以下至少一项相关的头部损伤:

[0136] 1.意识丧失>30分钟

[0137] 2.格拉斯哥昏迷量表(GCS)评分<13

[0138] 3.创伤后遗忘症1持续>24小时

[0139] 4.意识水平改变>24小时

[0140] 5.创伤性头部损伤的影像学证据,例如颅内出血和/或脑挫伤。

[0141] 由轻度创伤性头部损伤引起的持续性创伤后头痛的诊断标准可包括满足以下这两项的头部损伤:

[0142] 1.与以下任一项都不相关:

[0143] a)意识丧失>30分钟

[0144] b)格拉斯哥昏迷量表(GCS)评分<13

[0145] c)创伤后遗忘症持续>24小时

[0146] d)意识水平改变>24小时

[0147] e)创伤性头部损伤的影像学证据,例如颅内出血和/或脑挫伤

[0148] 2.在头部损伤后立即与以下一种或更多种症状和/或体征相关:

[0149] a)暂时性意识错乱、定向障碍或意识受损

[0150] b)头部损伤之前或之后立即丧失记忆力

[0151] c)表明轻度创伤性脑损伤的两种或更多种其他症状:恶心、呕吐、视觉障碍、头晕和/或眩晕、记忆力和/或注意力受损。

[0152] 创伤性头部损伤被定义为由对头部的外力作用导致的结构性或功能性损伤。这些包括用物体撞击头部或头部撞击物体、头部被外来物穿透、由爆炸或爆破产生的力、以及尚未确定的其他力。

[0153] 抗体的“可变区”是指单独或组合的抗体轻链的可变区或抗体重链的可变区。重链和轻链的可变区各自由通过也称为高变区的三个互补决定区 (CDR)连接的四个框架区(FR)组成。每条链中的CDR通过FR紧密靠近地保持在一起,并且与来自另一条链的CDR一起促成抗体的抗原结合位点的形成。至少有两种用于确定CDR的技术:(1)基于跨物种序列变异性的方法(即,Kabat等,Sequences of Proteins of Immunological Interest, (第5版,1991,National Institutes of Health,Bethesda MD));以及(2)基于抗原-抗体复合物的结晶学研究的方法(Al-lazikani等(1997)J.Molec. Biol.273:927-948))。本文使用的CDR可指通过任一种方法或通过这两种方法的组合限定的CDR。

[0154] 抗体的“恒定区”是指单独或组合的抗体轻链的恒定区或抗体重链的恒定区。

[0155] 与抗体或多肽“优先结合”或“特异性结合”(在本文中可互换使用)的表位是本领域中公知的术语,并且确定这样的特异性或优先结合的方法也是本领域中公知的。如果与分子与作为替代的细胞或物质反应或缔合相比,其更频繁、更迅速地、以更长的持续时间

和/或以更大的亲和力与特定细胞或物质反应或缔合，则认为其表现出“特异性结合”或“优先结合”。如果与抗体与其他物质结合相比，其以更大的亲和力(affinity)、亲合力(avidity)、更容易和/或以更长的持续时间结合，则其与靶标“特异性结合”或“优先结合”。例如，与CGRP表位特异性或优先结合的抗体是这样的抗体，与其与其他CGRP表位或非CGRP表位结合相比，其以更大的亲和力、亲合力、更容易和/或以更长的持续时间与该表位结合。通过阅读该定义也可以理解，例如，与第一靶标特异性或优先结合的抗体(或部分或表位)可以或可以不与第二靶标特异性或优先结合。因此，“特异性结合”或“优先结合”不一定必须要求(尽管其可包括)排他性结合。通常但不一定，提及结合意指优先结合。

[0156] 本文使用的“基本上纯的”是指至少50％纯(即，无污染物)、更优选至少90％纯、更优选至少95％纯、更优选至少98％纯、并且更优选至少 99％纯的材料。

[0157] “宿主细胞”包括单独细胞或细胞培养物，其可以是或已经成为用于并入多核苷酸插入片段之载体的接受者。宿主细胞包括单一宿主细胞的后代，并且由于天然的、意外的或故意的突变，后代可不一定与原始亲本细胞完全相同(在形态学或基因组DNA互补物中)。宿主细胞包括用本发明的多核苷酸体内转染的细胞。

[0158] 术语“Fc区”用于限定免疫球蛋白重链的C端区域。“Fc区”可以是天然序列Fc区或变体Fc区。虽然免疫球蛋白重链的Fc区的边界可能变化，但人IgG重链Fc区通常被限定为从第Cys226位或从第Pro230位的氨基酸残基延伸至其羧基末端。Fc区中残基的编号是如Kabat中的EU索引的编号。Kabat等，Sequences of Proteins of Immunological Interest，第5版. Public Health Service，National Institutes of Health，Bethesda，Md.，1991。免疫球蛋白的Fc区通常包含两个恒定结构域：CH2和CH3。

[0159] 本文使用的“Fc受体”和“FcR”描述与抗体的Fc区结合的受体。优选的 FcR是天然序列人FcR。此外，优选的FcR是结合IgG抗体(γ受体)的 FcR，并且包括FcγRI、FcγRII和FcγRIII亚类的受体，包括这些受体的等位基因变体和选择性剪接形式。FcγRII受体包括具有主要在其胞质结构域不同的类似氨基酸序列的FcγRIIA(“激活受体”)和FcγRIIB(“抑制受体”)。FcR在Ravetch和Kinet，1991，Ann.Rev.Immunol.，9：457-92；Capel等，1994，Immunomethods，4：25-34；以及de Haas等，1995，J.Lab.Clin.Med.， 126：330-41中综述。“FcR”还包括负责将母体IgG转移至胎儿的新生儿受体FcRn(Guyer等，1976，J.Immunol.，117：587；和Kim等，1994，J.Immunol.， 24：249)。

[0160] “补体依赖性细胞毒性”和“CDC”是指在补体存在下裂解靶标。补体激活途径由补体系统的第一组分(C1q)与和同源抗原复合的分子(例如，抗体)结合而启动。为了评估补体活化，可进行CDC测定，例如如 Gazzano-Santoro等，J.Immunol.Methods，202：163(1996)中所述。

[0161] “功能性Fc区”具有天然序列Fc区的至少一种效应功能。示例性的“效应功能”包括C1q结合；补体依赖性细胞毒性(CDC)；Fc受体结合；抗体依赖性细胞介导的细胞毒性(ADCC)；吞噬；细胞表面受体(例如，B 细胞受体；BCR)的下调等。这样的效应功能通常需要Fc区与结合结构域(例如，抗体可变结构域)组合，并且可使用本领域中已知用于评价这样的抗体效应功能的多种测定来评估。

[0162] “天然序列Fc区”包含与天然存在的Fc区的氨基酸序列相同的氨基酸序列。“变体Fc区”包含由于至少一个氨基酸修饰而不同于天然序列Fc 区但仍保留天然序列Fc区的至

少一种效应功能的氨基酸序列。优选地，与天然序列Fc区或亲本多肽的Fc区相比，变体Fc区在天然序列Fc区中或在亲本多肽的Fc区中具有至少一个氨基酸替换，例如约1至约10个氨基酸替换，并且优选约1至约5个氨基酸替换。本文中的变体Fc区将优选与天然序列Fc区和/或与亲本多肽的Fc区具有至少约80％的序列同一性，并且最优选与其具有至少约90％的序列同一性，更优选与其具有至少约95％、至少约96％、至少约97％、至少约98％、至少约99％的序列同一性。

[0163] 本文使用的“抗体依赖性细胞介导的细胞毒性”和“ADCC”是指细胞介导的反应，其中表达Fc受体(FcR)的非特异性细胞毒性细胞(例如，自然杀伤(NK)细胞、中性粒细胞和巨噬细胞)识别靶细胞上结合的抗体并随后引起靶细胞裂解。目的分子的ADCC活性可使用体外ADCC测定来评估，例如美国专利No.5,500,362或5,821,337中所述的体外ADCC测定。用于这样的测定的可用效应细胞包括外周血单个核细胞(peripheral blood mononuclear cell，PBMC)和NK细胞。作为替代或补充，目的分子的ADCC活性可在体内评估，例如在动物模型(例如Clynes等，1998，PNAS (USA)，95:652-656中公开的动物模型)中评估。

[0164] 本文使用的“预防”是在尚未患有(持续性)PTH的对象中阻止(持续性)PTH发生或存在的方法。本文使用的“治疗”是用于获得有益或期望的临床结果的方法。出于本发明的目的，有益或期望的临床结果包括但不限于以下一项或更多项：改善创伤后头痛的任何方面，包括减轻严重程度、缓解疼痛强度和其他相关症状、降低复发的频率、提高患有创伤后头痛的那些的生命质量，以及降低治疗创伤后头痛所需的其他药物的剂量。

[0165] “降低创伤后头痛的发病率”意指以下中的任一项：降低严重程度(其可包括降低对这种病症通常使用的其他药物和/或治疗的需要和/或量(例如，暴露))，所述其他药物和/或治疗包括例如麦角胺、双氢麦角胺或曲普坦类)、持续时间和/或频率(包括例如延迟或提高个体中到下次发作的时间)。如本领域技术人员所理解的，个体可在其针对治疗的响应方面有所不同，并且因此例如“在个体中降低创伤后头痛发病率的方法”反映基于以下合理预期来施用抗CGRP拮抗剂抗体：这样的施用可能会在该特定个体中引起这样的发病率降低。

[0166] “改善”创伤后头痛或者创伤后头痛的一种或更多种症状意指与未施用抗CGRP拮抗剂抗体相比减轻或改善创伤后头痛的一种或更多种症状。“改善”还包括缩短或降低症状的持续时间。

[0167] 本文使用的“控制创伤后头痛”是指在个体中维持或降低创伤后头痛的一种或更多种症状的严重程度或持续时间，或者创伤后头痛发作的频率 (与治疗前的水平相比)。例如，与治疗前的水平相比，头部和/或颈部疼痛的持续时间或严重程度或者发作频率在个体中降低至少约10％、20％、 30％、40％、50％、60％或70％中的任一种。

[0168] 本文使用的“头痛小时”是指对象经历头痛的小时。头痛小时可表示为整小时(例如，1个头痛小时、2个头痛小时、3个头痛小时等)或表示为整小时和部分小时(例如0.5个头痛小时、1.2个头痛小时、2.67个头痛小时等)。一个或更多个头痛小时可相对于特定的时间间隔进行描述。例如，“每日头痛小时”可指对象在一天的间隔(例如，24小时的时间)内经历的头痛小时数。在另一个实例中，“每周头痛小时”可指对象在一周的间隔(例如，7天的时间)内经历的头痛小时数。可理解的是，一周的间隔可以或可以不对应于日历周。在另一个实例中，“每月头痛小时”可指对象在一个月的间隔内经历的头痛小时数。可理解的是，一个

月的间隔(例如,28、29、30或31天的时间)可根据特定月份而在天数方面变化,并且可以或可以不对应于日历月。在另一个实例中,“每年头痛小时”可指对象在一年的间隔内经历的头痛小时数。可理解的是,一年的间隔(例如, 365或366天的时间)可根据特定年份而在天数方面变化,并且可以或可以不对应于日历年。

[0169] 本文使用的“头痛日”是指对象经历头痛的天。头痛日可表示为整日 (例如,1个头痛日、2个头痛日、3个头痛日等)或表示为整日和部分日(例如,0.5个头痛日、1.2个头痛日、2.67个头痛日等)。一个或更多个头痛日可相对于特定的时间间隔进行描述。例如,“每周头痛日”可指对象在一周的间隔(例如,7天的时间)内经历的头痛日数。可理解的是,一周的间隔可以或可以不对应于日历周。在另一个实例中,“每月头痛日”可指对象在一个月的间隔内经历的头痛日数。可理解的是,一个月的间隔 (例如,28、29、30或31天的时间)可根据特定月份而在天数方面变化,并且可以或可以不对应于日历月。在另一个实例中,“每年头痛日”可指对象在一年的间隔内经历的头痛日数。可理解的是,一年的间隔(例如,365 或366天的时间)可根据特定年份而在天数方面变化,并且可以或可以不对应于日历年。

[0170] 本文使用的“延迟”创伤后头痛的发生意指拖延、阻碍、减慢、延缓、稳定和/或推迟疾病的进展。这种延迟的时间长度可以有所不同,这取决于疾病史和/或正在接受治疗的个体。对于本领域技术人员显而易见的是,充分或显著的延迟实际上可涵盖预防,因为个体不发生创伤后头痛。“延迟”症状发生的方法是与未使用该方法相比,在给定时间框架内降低发生症状的概率和/或在给定时间框架内降低症状程度的方法。这样的比较通常基于使用统计学上显著数量对象的临床研究。

[0171] 创伤后头痛的“发生”或“进展”意指该病症的初始表现和/或随后的进展。创伤后头痛的发生可使用本领域中公知的标准临床技术来检测和评估。然而,发生也是指可无法检出的进展。出于本公开内容的目的,发生或进展是指症状的生物学过程。“发展”包括发生、复发和发作。本文使用的创伤后头痛的“发作”或“发生”包括初始发作和/或复发。

[0172] 本文使用的药物、化合物或药物组合物的“有效剂量”或“有效量”是足以实现有益或期望结果的量。对于预防性使用,有益或期望的结果包括例如以下的结果:消除或降低风险、减轻严重程度或延迟疾病发作,包括疾病的生物化学、组织学和/或行为症状,其在疾病的发生期间出现的并发症和中间病理学表型。对于治疗性使用,有益或期望的结果包括例如以下的临床结果:降低疼痛强度、持续时间或创伤后头痛发作的频率;以及减轻由创伤后头痛引起的一种或更多种(生物化学、组织学和/或行为的) 症状,包括其在疾病发生期间出现的并发症和中间病理学表型;提高患有疾病的那些的生命质量;降低治疗疾病所需的其他药物的剂量;增强另外药物的作用;和/或延迟患者的疾病进展。有效剂量可在一次或更多次施用中施用。出于本公开内容的目的,药物、化合物或药物组合物的有效剂量是足以直接或间接地实现预防性或治疗性治疗的量。如在临床背景下所理解的,药物、化合物或药物组合物的有效剂量可以或可以不与其他药物、化合物或药物组合物联合实现。因此,可在施用一种或更多种治疗剂的情况下考虑“有效剂量”,并且如果与一种或更多种其他药剂联合可实现或实现期望的结果,则可认为单一药剂以有效量给予。

[0173] “个体”或“对象”是哺乳动物,更优选人。哺乳动物还包括但不限于:农场动物、运动动物、宠物、灵长类、马、狗、猫、小鼠和大鼠。

[0174] 本文使用的“载体”意指能够在宿主细胞中递送并且优选地表达一种或更多种目

的基因或目的序列的构建体。载体的实例包括但不限于病毒载体、裸DNA或RNA表达载体、质粒、黏粒或噬菌体载体、与阳离子缩合剂缔合的DNA或RNA表达载体、包封在脂质体中的DNA或RNA表达载体，以及某些真核细胞，例如生产细胞(producer cell)。

[0175] 本文使用的“表达控制序列”意指指导核酸转录的核酸序列。表达控制序列可以是启动子，例如组成型或诱导型启动子，或增强子。表达控制序列与待转录的核酸序列可操作地连接。

[0176] 本文使用的“可药用载体”或“可药用赋形剂”包括当与活性成分组合时允许该成分保留生物活性并且不与对象的免疫系统反应的任何材料。实例包括但不限于任何标准药用载体，例如磷酸缓冲盐水溶液、水、乳剂(例如油/水乳剂)、以及多种类型的润湿剂。用于气雾剂或肠胃外施用的优选稀释剂是磷酸缓冲盐水或生理盐水(0.9％)。包含此类载体的组合物通过公知的常规方法配制(参见例如Remington′s Pharmaceutical Sciences，第18版，A.Gennaro，编辑，Mack Publishing Co.，Easton，PA，1990；和 Remington，The Science and Practice of Pharmacy第20版Mack Publishing，2000)。

[0177] 本文使用的术语“k_{on}”旨在指抗体与抗原缔合的速率常数。

[0178] 本文使用的术语“k_{off}”旨在指抗体从抗体/抗原复合物解离的速率常数。

[0179] 本文使用的术语“K_D”旨在指抗体-抗原相互作用的平衡解离常数。

[0180] A.用于预防、治疗或减轻创伤后头痛和/或与创伤后头痛相关的至少一种继发症状的方法

[0181] 在一个方面，本发明提供了在对象中预防、治疗(持续性)创伤后头痛或降低其发病率的方法。在另一方面，本发明提供了在对象中治疗与创伤后头痛相关的至少一种继发症状或降低其发病率的方法。在一些实施方案中，所述方法包括向个体施用有效量的来自于调节CGRP途径的抗体的抗体或多肽(例如，单克隆抗CGRP拮抗剂抗体)。

[0182] 在另一方面，本发明提供了用于在个体中预防、改善、控制(持续性) 创伤后头痛或与(持续性)创伤后头痛相关的症状(例如，腹泻、光敏性、发热、颈强直(stiff neck)、恶心、认知受损和/或呕吐)、降低其发病率或延迟其发生或进展的方法，其包括向个体施用有效量的与可用于预防、治疗或减轻(持续性)创伤后头痛的至少一种另外的药剂组合的调节CGRP 途径的抗体或抗CGRP拮抗剂抗体。

[0183] 这样的另外的药剂包括但不限于5-HT激动剂和NSAID。例如，抗体和至少一种另外的药剂可同时施用，即其可在足够接近的时间内给予以允许其各自的治疗效果重叠。例如，与在不存在其他药剂的情况下施用这些药剂中的任一种相比，与抗CGRP抗体组合施用的5-HT激动剂或NSAID 的量应足以降低患者中(持续性)创伤后头痛复发的频率或产生更加持久的效力。

[0184] 可与抗CGRP拮抗剂抗体组合施用的另外的药剂的另一些非限制性实例包括以下一种或更多种：

[0185] (i)阿片类镇痛剂，例如吗啡、海洛因、氢吗啡酮、氧吗啡酮、左啡诺、左洛啡烷、美沙酮、哌替啶、芬太尼、可卡因、可待因、双氢可待因、羟考酮、氢可酮、丙氧芬、纳美芬、烯丙吗啡、纳洛酮、纳曲酮、丁丙诺啡、布托啡诺、纳布啡或喷他佐辛；

[0186] (ii)非甾体抗炎药(NSAID)，例如阿司匹林、双氯芬酸、二氟尼柳、依托度酸、芬布芬、非诺洛芬、氟苯柳、氟比洛芬、布洛芬、吲哚美辛、酮洛芬、酮咯酸、甲氯芬那酸、甲芬那

酸、萘丁美酮、萘普生、奥沙普秦、保泰松、吡罗昔康、舒林酸、托美丁或佐美酸、环氧合酶-2(COX-2)抑制剂(塞来昔布、罗非昔布、美洛昔康、JTE-522、L-745,337、NS398)、或其可药用盐;

[0187] (iii)巴比妥类镇静剂,例如异戊巴比妥、阿普比妥、布塔巴比妥 (butabarbital)、布他比妥(butabital)、甲苯比妥、美沙比妥、美索比妥、戊巴比妥、苯巴比妥、司可巴比妥、他布比妥、硫戊巴比妥(theamylal) 或硫喷妥,或其可药用盐;

[0188] (iv)巴比妥类镇痛剂,例如布他比妥或其可药用盐或者包含布他比妥的组合物。

[0189] (v)具有镇静作用的苯二氮䓬类,例如氯氮䓬(chlordiazepoxide、氯䓬酸盐(clorazepate)、地西泮、氟西泮、劳拉西泮、奥沙西泮、替马西泮或三唑仑,或其可药用盐;

[0190] (vi)具有镇静作用的H_1拮抗剂,例如苯海拉明、吡拉明、异丙嗪、氯苯那敏或氯环嗪,或其可药用盐;

[0191] (vii)镇静剂,例如格鲁米特、甲丙氨酯、甲喹酮或氯醛比林,或其可药用盐;

[0192] (viii)骨骼肌松弛剂,例如巴氯芬、卡立普多、氯唑沙宗、环苯扎林、美索巴莫或者欧弗瑞那定(orphrenadine),或其可药用盐;

[0193] (ix)NMDA受体拮抗剂,例如右美沙芬((+)-3-羟基-N-甲基吗啡喃)或其代谢物右啡烷((+)-3-羟基-N-甲基吗啡喃)、氯胺酮、美金刚、吡咯并喹啉醌或顺式-4-(膦酰基甲基)-2-哌啶羧酸,或其可药用盐;

[0194] (x)α-肾上腺素能剂,例如多沙唑嗪、坦洛新、可乐定或4-氨基-6,7-二甲氧基-2-(5-甲磺酰氨基-1,2,3,4-四氢异喹啉-2-基)-5-(2-吡啶基)喹唑啉;

[0195] (xi)三环类抗抑郁剂,例如地昔帕明、丙咪嗪、阿米替林(amytriptiline) 或去甲替林(nortriptiline);

[0196] (xii)抗惊厥剂,例如卡马西平或丙戊酸盐(valproate);

[0197] (xiii)速激肽(tachykinin,NK)拮抗剂,特别是NK-3、NK-2或NK-1 拮抗剂,例如(αR,9R)-7-[3,5-双(三氟甲基)苄基]-8,9,10,11-四氢-9-甲基-5-(4- 甲基苯基)-7H-[1,4]二氮芳辛并[2,1-g][1,7]萘啶-6-13-二酮(TAK-637)、 5-[[(2R,3S)-2-[(1R)-1-[3,5-双(三氟甲基)苯基]乙氧基-3-(4-氟苯基)-4-吗啉基]甲基]-1,2-二氢-3H-1,2,4-三唑-3-酮(MK-869)、拉奈匹坦(lanepitant)、达匹坦(dapitant)或3-[[2-甲氧基-5-(三氟甲氧基)苯基]甲基氨基]-2-苯基- 哌啶(2S,3S);

[0198] (xiv)毒蕈碱拮抗剂,例如奥昔布宁(oxybutin)、托特罗定、丙哌维林、曲司氯铵(tropsium chloride)或达非那新(darifenacin);

[0199] (xv)COX-2抑制剂,例如塞来昔布、罗非昔布或伐地昔布;

[0200] (xvi)非选择性COX抑制剂(优选具有GI保护作用),例如硝基氟吡洛芬(HCT-1026);

[0201] (xvii)煤焦油镇痛剂,特别是对乙酰氨基酚;

[0202] (xviii)精神安定剂(neuroleptic),例如氟哌利多;

[0203] (xix)香草素受体激动剂(例如,树脂毒素(resinferatoxin))或拮抗剂(例如辣椒平(capsazepine));

[0204] (xx)β-肾上腺素能剂,例如普萘洛尔;

[0205] (xxi)局部麻醉剂,例如美西律;

[0206] (xxii)皮质类固醇,例如地塞米松;

[0207] (xxiii)5-羟色胺受体激动剂或拮抗剂;

[0208] (xxiv)胆碱能(烟碱)镇痛剂;

[0209] (xxv)曲马朵(商标);

[0210] (xxvi)PDEV抑制剂,例如西地那非、伐地那非或他达拉非;

[0211] (xxvii)α-2-δ配体,例如加巴喷丁或普瑞巴林;

[0212] (xxviii)大麻素类(canabinoid);以及

[0213] (xxix)抗抑郁剂,例如阿米替林(Elavil)、曲唑酮(Desyrel)和丙咪嗪(Tofranil);或抗惊厥剂,例如苯妥英(Dilantin)或卡马西平(Tegretol)。

[0214] 本领域技术人员将能够确定与抗CGRP抗体组合使用的特定药剂的合适剂量。例如,舒马普坦可以以约0.01至约300mg的剂量施用。在一些情况下,舒马普坦可以以下剂量施用:约2mg至约300mg,例如约5mg 至约250mg、约5mg至约200mg、约5mg至约100mg、约5mg至约 50mg、或约5mg至约25mg。当非肠胃外施用时,舒马普坦的典型剂量为约25至约100mg,通常优选约50mg,例如约45mg、约55mg或约 60mg。当舒马普坦肠胃外施用时,优选的剂量为约6mg,例如约5mg、约7mg或约8mg。然而,这些剂量可根据本领域标准的方法而变化,使得其针对特定患者或针对特定组合治疗而被优化。此外,例如,塞来昔布可以以下量施用:50至500mg,例如约50mg至约400mg、约50mg至约300mg、约50mg至约200mg、约50mg至约100mg、约100mg至约400mg、或约200mg至约300mg。

[0215] 在另一方面,本公开内容提供了在对象中预防、治疗(持续性)创伤后头痛或降低其发病率的方法,其包括向所述对象施用调节CGRP途径的单克隆抗体(例如,单克隆抗CGRP拮抗剂抗体)。在一些实施方案中,在多天中的每一天施用的单克隆抗体的量可以是0.1mg至5000mg、1mg 至5000mg、10mg至5000mg、100mg至5000mg、1000mg至5000mg、 0.1mg至4000mg、1mg至4000mg、10mg至4000mg、100mg至4000 mg、1000mg至4000mg、0.1mg至3000mg、1mg至3000mg、10mg 至3000mg、100mg至3000mg、1000mg至3000mg、0.1mg至2000mg、 1mg至2000mg、10mg至2000mg、100mg至2000mg、1000mg至2000 mg、0.1mg至1000mg、1mg至1000mg、10mg至1000mg或100mg 至1000mg。在一些实施方案中,该量为约225mg至约1000mg,例如约675mg或约900mg。一个示例性给药方案包括皮下施用约675mg的初始抗体剂量,随后皮下施用约225mg的月抗体剂量持续约2个月,例如约 3个月、4个月、5个月、6个月或12个月。另一个给药方案包括以约60 分钟的输注静脉内施用约900mg的初始抗体剂量,随后每季度以约60分钟的输注静脉内施用约900mg的剂量持续一年、两年、三年、四年或五年。然而,另一些剂量方案可以是可用的,这取决于从业者希望实现的药动学衰减的模式。在一些实施方案中,初始剂量和一个或更多个另外的剂量以相同的方式施用,例如皮下或静脉内施用。在一些实施方案中,所述一个或更多个另外的剂量以与初始剂量不同的方式施用,例如初始剂量可静脉内施用并且所述一个或更多个另外的剂量可皮下施用。

[0216] 在另一方面,本公开内容提供了在对象中预防、治疗(持续性)创伤后头痛或降低其发病率的方法,其包括以调节CGRP途径的量向所述对象施用单次剂量的单克隆抗体(例如,单克隆抗CGRP拮抗剂抗体)。在一些实施方案中,单次剂量可以是以下抗体量:0.1mg至5000mg、1mg至 5000mg、10mg至5000mg、100mg至5000mg、1000mg至5000mg、 0.1mg至4000mg、1mg至4000mg、10mg至4000mg、100mg至4000 mg、1000mg至4000mg、0.1mg至3000mg、1mg至

3000mg、10mg 至3000mg、100m至3000mg、1000mg至3000mg、0.1mg至2000mg、 1mg至2000mg、10mg至2000mg、100mg至2000mg、1000mg至2000 mg、0.1mg至1000mg、1mg至1000mg、10mg至1000mg或100mg 至1000mg。在一些实施方案中，单次剂量可以是225mg至约1000mg、例如约675mg或约900mg的抗体量。

[0217] 在另一方面，本公开内容提供了在对象中预防、治疗(持续性)创伤后头痛或降低其发病率的方法，其包括以调节CGRP途径的量向所述对象施用月剂量的单克隆抗体(例如，单克隆抗CGRP拮抗剂抗体)。在一些实施方案中，单次剂量可以是以下抗体量：0.1mg至5000mg、1mg至 5000mg、10mg-5000mg、100mg至5000mg、1000mg至5000mg、0.1 mg至4000mg、1mg至4000mg、10mg至4000mg、100mg至4000mg、 1000mg至4000mg、0.1mg至3000mg、1mg至3000mg、10mg至3000 mg、100mg至3000mg、1000mg至3000mg、0.1mg至2000mg、1mg 至2000mg、10mg至2000mg、100mg至2000mg、1000mg至2000mg、 0.1mg至1000mg、1mg至1000mg、10mg至1000mg或100mg至1000 mg。在一些实施方案中，月剂量可以是约225mg至约1000mg、例如约675mg或约900mg的抗体量。一个示例性给药方案包括皮下施用约675 mg的初始抗体剂量，随后皮下施用约225mg的月抗体剂量持续约2个月，例如约3个月、4个月、5个月、6个月或12个月。另一个给药方案包括以约60分钟的输注静脉内施用约900mg的初始抗体剂量，随后每季度以约60分钟的输注静脉内施用约900mg的剂量持续一年、两年、三年、四年或五年。然而，另一些剂量方案可以是可用的，这取决于从业者希望实现的药动学衰减的模式。在一些实施方案中，初始剂量和一个或更多个另外的剂量以相同的方式施用，例如皮下或静脉内施用。在一些实施方案中，所述一个或更多个另外的剂量以与初始剂量不同的方式施用，例如初始剂量可静脉内施用并且一个或更多个另外的剂量可皮下施用。

[0218] 在另一方面，本公开内容提供了降低对象经历的每月头痛小时数的方法，其包括向所述对象施用一定量的调节CGRP途径的单克隆抗体(例如，单克隆抗CGRP拮抗剂抗体)。在一些实施方案中，单克隆抗体的量可在单次剂量、月剂量或季度剂量之后有效地使每月头痛小时数降低至少0.1、 1、5、10、15、20、25、30、35、40、45、50、55、60、65、70、75、80、 85、90、95、100或更多个头痛小时。在一些实施方案中，单克隆抗体的量可以在单次剂量、月剂量或季度剂量之后有效地使每月头痛小时数降低至少20个头痛小时。在一些实施方案中，单克隆抗体的量可有效地使每月头痛小时数降低至少40、45、50、55、60、65、70、75、80、85、90、 95、100、105、110、115、120、125个或更多个头痛小时。在一些实施方案中，单克隆抗体的量可在单次剂量之后有效地使每月头痛小时数降低至少0.1％、1％、2％、3％、4％、5％、6％、7％、8％、9％、10％、15％、20％、 25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、 85％、90％、95％、99％或更多。在一些实施方案中，单克隆抗体的量可在单次剂量、月剂量或季度剂量之后有效地使每月头痛小时数降低至少 15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、 75％、80％、85％、90％、95％、99％或更多。

[0219] 在另一方面，本公开内容提供了降低对象经历的每月头痛日数的方法，其包括向所述对象施用一定量的调节CGRP途径的单克隆抗体(例如，单克隆抗CGRP拮抗剂抗体)。在一些实施方案中，单克隆抗体的量可在单次剂量之后有效地使每月头痛日数降低至少3、4、5、6、7、8、9、10、 11、12、13、14、15、16、17、18、19、20个或更多个头痛日。在一些实施方案中，单克隆抗体的量可在月剂量或季度剂量之后有效地使每月头痛日数降低至少3、4、5、6、7、

8、9、10、11、12、13、14、15、16、17、 18、19、20个或更多个头痛日。在一些实施方案中，单克隆抗体的量可在单次剂量、月剂量或季度剂量之后有效地使每月头痛日数降低至少 0.1%、1%、2%、3%、4%、5%、6%、7%、8%、9%、10%、15%、20%、 25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、 85%、90%、95%、99%或更多。

[0220] 在另一方面，本公开内容提供了降低对象中抗头痛药物的使用的方法，其包括向所述对象施用调节CGRP途径的单克隆抗体(例如，单克隆抗CGRP拮抗剂抗体)。在一些实施方案中，单克隆抗体的量可有效地使对象对抗头痛药物的每日、每月、每季度和/或每年使用降低至少0.1%、 1%、2%、3%、4%、5%、6%、7%、8%、9%、10%、15%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、 90%、95%、99%或更多。在一些实施方案中，单克隆抗体的量可以有效地使对象对抗头痛药物的每月使用降低至少15%、20%、25%、30%、35%、 40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、95%、 99%或更多。抗头痛药物可以是本文中所述的任何类型的抗头痛药物。抗头痛药物的非限制性实例包括例如5-HT1激动剂(和作用于其他5-HT1 位点的激动剂)、曲普坦类(例如，舒马普坦、佐米曲普坦、那拉曲普坦、利扎曲普坦、依来曲普坦、阿莫曲普坦、夫罗曲普坦)、麦角生物碱类(例如，酒石酸麦角胺、马来酸麦角新碱和甲磺酸二氢麦角碱(例如，双氢麦角考宁、双氢麦角汀、双氢麦角隐亭和甲磺酸双氢麦角胺(DHE 45))和非甾体抗炎药(NSAID)(例如，阿司匹林、双氯芬酸、二氟尼柳、依托度酸、芬布芬、非诺洛芬、氟苯柳、氟比洛芬、布洛芬、吲哚美辛、酮洛芬、酮咯酸、甲氯芬那酸、甲芬那酸、萘丁美酮、萘普生、奥沙普秦、保泰松、吡罗昔康、舒林酸、托美汀或佐美酸、环氧合酶-2(COX-2)抑制剂(塞来昔布、罗非昔布、美洛昔康、JTE-522、L-745，337、NS398)、或其可药用盐)、阿片类(例如，羟考酮)和β-肾上腺素能拮抗剂(例如，普萘洛尔)。

[0221] 对于本文中所述的所有方法，提及抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)还包括包含一种或更多种这些药剂的组合物。因此，可根据涉及本文中描述的抗体的方法使用这样的组合物。这些组合物还可包含合适的赋形剂，例如本文中其他部分所述的可药用赋形剂。本发明可单独使用或与其他常规治疗方法组合使用。

[0222] 本文中所述的抗体(例如，单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)可以以任何治疗剂量、通过任何合适的途径并以任何合适的制剂施用于个体或对象。对于本领域技术人员应明显的是，本文中描述的实例并非旨在进行限制，而是为了举例说明可用技术。因此，在一些实施方案中，可根据已知方法，例如静脉内施用，例如通过推注或通过在例如以下时间内连续输注来将本文中所述的抗体施用于对象：约 10分钟、约20分钟、约30分钟、约40分钟、约50分钟、约60分钟、约90分钟、约120分钟、约180分钟或约240分钟。本文中所述的抗体还可通过以下途径施用于对象：皮下、肌内、腹膜内、脑脊髓内、关节内、舌下、动脉内、滑膜内、通过吹入(insufflation)、鞘内、经口、吸入、鼻内(例如，具有或不具有吸入)、口含(buccal)、经直肠、经皮、心内、骨内、皮内、经黏膜、经阴道、玻璃体内、关节外周、局部、经表皮 (epicutaneous)或表面途径。施用可以是全身性的，例如静脉内施用，或局部的。用于液体制剂的可商购雾化器(包括喷射雾化器和超声雾化器) 可用于施用。液体制剂可以直接雾化，并且冻干粉末可在重构后雾化。或者，可使用氟碳化合物(fluorocarbon)制剂和计量吸入器来将本文中所述的抗体气雾化，或者将其作为冻干和经

研磨粉末吸入。

[0223] 在一些实施方案中,可通过位点特异性或靶向局部递送技术来施用本文中所述的抗体。位点特异性或靶向局部递送技术的一些实例包括抗体的多种可植入贮库源或局部递送导管,例如输注导管、留置导管或针导管、合成移植物、外膜包裹物(adventitial wrap)、分流器(shunt)和支架或其他可植入装置、位点特异性载体、直接注射或直接施加。参见例如PCT 公开No.WO 00/53211和美国专利No.5,981,568,其在此通过引用整体并入。

[0224] 本文中所述的抗体的多种制剂可以用于施用。在一些实施方案中,可施用纯抗体。在一些实施方案中,抗体和可药用赋形剂可在多种制剂中。可药用赋形剂是本领域中已知的,并且是促进药理学有效物质的施用的相对惰性的物质。例如,赋形剂可赋予形式或稠度,或充当稀释剂。合适的赋形剂包括但不限于:稳定剂、润湿剂和乳化剂、用于不同渗量(osmolarity)的盐、包封剂、缓冲剂和皮肤渗透增强剂。赋形剂以及用于肠胃外和非肠胃外药物递送的制剂在Remington,The Science and Practice of Pharmacy第20版Mack Publishing(2000)中阐述。

[0225] 在一些实施方案中,可将这些药剂(包括本文中所述的抗体)配制成通过注射(例如,静脉内、皮下、腹膜内、肌内等)施用。因此,这些药剂可与可药用载剂组合,所述可药用载剂例如盐水、林格液(Ringer's solution)、右旋糖溶液等。特定的剂量方案(即剂量、时间和重复)将取决于特定个体和该个体的病史(medical history)。

[0226] 在一些实施方案中,这些药剂(包括本文中所述的抗体)可配制成用于外周施用。这样的制剂可通过任何合适的外周途径(包括静脉内和皮下) 外周施用。准备用于外周施用的药剂可包括不经中枢、经脊髓、鞘内递送或直接递送到CNS中的物质、药物和/或抗体。外周施用途径的非限制性实例包括以下途径:经口、舌下、口含、表面、经直肠、经由吸入、经皮、皮下、静脉内、动脉内、肌内、心内、骨内、皮内、腹膜内、经黏膜、经阴道、玻璃体内、关节内、关节外周、局部或经表皮。

[0227] 根据本公开内容使用的抗体的治疗性制剂可通过将具有期望纯度的抗体与任选的可药用载体、赋形剂或稳定剂(Remington,The Science and Practice of Pharmacy第20版Mack Publishing(2000))混合来制备以供储存和/或使用,并且其在一些情况下可以是冻干制剂或水溶液的形式。可接受的载体、赋形剂或稳定剂在使用的剂量和浓度下对接受者无毒。抗体的治疗性制剂可包含一种或更多种可药用载体、赋形剂或稳定剂,这些物质的非限制性实例包括:缓冲剂,例如磷酸盐、柠檬酸盐和其他有机酸;盐,例如氯化钠;抗氧化剂,包括抗坏血酸和甲硫氨酸;防腐剂(例如十八烷基二甲基苄基氯化铵;六甲双铵氯化物(hexamethonium chloride);苯扎氯铵、苄索氯铵;酚、丁基或苄基醇;对羟基苯甲酸烷基酯,例如对羟基苯甲酸甲酯或对羟基苯甲酸丙酯;儿茶酚;间苯二酚;环己醇;3-戊醇;以及间甲酚);低分子量(少于约10个残基)多肽;蛋白质,例如血清白蛋白、明胶或免疫球蛋白;亲水性聚合物,例如聚乙烯吡咯烷酮;氨基酸(例如,浓度为0.1mM至100mM、0.1mM至1mM、0.01mM至 50mM、1mM至50mM、1mM至30mM、1mM至20mM、10mM至 25mM),例如甘氨酸、谷氨酰胺、甲硫氨酸、天冬酰胺、组氨酸、精氨酸或赖氨酸;单糖、二糖和其他碳水化合物,包括葡萄糖、甘露糖或糊精;螯合剂(例如,浓度为0.001mg/mL至1mg/mL、0.001mg/mL至1mg/mL、0.001mg/mL至0.1mg/mL、0.001mg/mL至0.01mg/mL、0.01mg/mL至 0.1mg/mL),例如EDTA(例如EDTA二钠二水合物);糖(例如,浓度为1mg/mL至500mg/mL、10mg/mL至200mg/mL、10mg/mL

至100 mg/mL、50mg/mL至150mg/mL)，例如蔗糖、甘露糖醇、海藻糖或山梨糖醇；成盐反离子，例如钠；金属络合物(例如Zn-蛋白质络合物)；和/ 或非离子表面活性剂(例如，浓度为0.01mg/mL至10mg/mL、0.01mg/mL 至1mg/mL、0.1mg/mL至1mg/mL、0.01mg/mL至0.5mg/mL)，例如 TWEEN™(例如，聚山梨酯(例如，聚山梨酯20、聚山梨酯40、聚山梨酯60、聚山梨酯80))、PLURONICS™或聚乙二醇(PEG)。

[0228] 可在多种物理特性中的任一种方面来表征抗体制剂。例如，液体抗体制剂可具有用于治疗效力、安全性和储存的任何合适的pH。例如，液体抗体制剂的pH可以是pH 4至约pH 9、约pH 5至约pH 8、约pH 5至约 pH 7或约pH 6至约pH 8。在一些实施方案中，液体抗体制剂的pH可以是约3.0、3.5、4.0、4.5、5.0、5.5、6.0、6.5、7.0、7.5、8.0、8.5、9.0、 9.5或约10或者更高或更低。

[0229] 在另一个实例中，液体抗体制剂可具有用于治疗效力、安全性和储存的任何合适的黏度。例如，在25℃下，液体抗体制剂的黏度可以是约0.5 厘泊(cP)至约100cP、约1cP至约50cP、约1cP至约20cP、约1cP 至约15cP、或约5cP至约15cP。在一些实施方案中，在25℃C下，液体抗体制剂的黏度可以是约0.5cP、1cP、1.2cP、1.4cP、1.6cP、1.8cP、 2.0cP、2.2cP、2.4cP、2.6cP、2.8cP、3.0cP、3.2cP、3.4cP、3.6cP、 3.8cP、4.0cP、4.2cP、4.4cP、4.6cP、4.8cP、5.0cP、5.2cP、5.4cP、 5.6cP、5.8cP、6.0cP、6.2cP、6.4cP、6.6cP、6.8cP、7.0cP、7.2cP、 7.4cP、7.6cP、7.8cP、8.0cP、8.2cP、8.4cP、8.6cP、8.8cP、9.0cP、 9.2cP、9.4cP、9.6cP、9.8cP、10.0cP、10.2cP、10.4cP、10.6cP、10.8 cP、11.0cP、11.2cP、11.4cP、11.6cP、11.8cP、12.0cP、12.2cP、12.4cP、 12.6cP、12.8cP、13.0cP、13.2cP、13.4cP、13.6cP、13.8cP、14.0cP、 14.2cP、14.4cP、14.6cP、14.8cP或约15.0cP，或者黏度可更高或更低。

[0230] 在另一个实例中，液体抗体制剂可具有用于治疗效力、安全性和储存的任何合适的电导率。例如，液体抗体制剂的电导率可以是约0.1毫西门子/厘米(mS/cm)至约15mS/cm、0.1mS/cm至10mS/cm、0.1mS/cm至 5mS/cm、0.1mS/cm至2mS/cm或0.1mS/cm至1.5mS/cm。在一些实施方案中，液体抗体制剂的电导率可以是0.19mS/cm、0.59mS/cm、1.09 mS/cm、1.19mS/cm、1.29mS/cm、1.39mS/cm、1.49mS/cm、1.59mS/cm、 1.69mS/cm、1.79mS/cm、1.89mS/cm、1.99mS/cm、2.09mS/cm、2.19 mS/cm、2.29mS/cm、2.39mS/cm、2.49mS/cm、2.59mS/cm、2.69mS/cm、 2.79mS/cm、2.89mS/cm、2.99mS/cm、3.09mS/cm、3.19mS/cm、3.29 mS/cm、3.39mS/cm、3.49mS/cm、3.59mS/cm、3.69mS/cm、3.79mS/cm、 3.89mS/cm、3.99mS/cm、4.09mS/cm、4.19mS/cm、4.29mS/cm、4.39 mS/cm、4.49mS/cm、4.59mS/cm、4.69mS/cm、4.79mS/cm、4.89mS/cm、 4.99mS/cm、5.09mS/cm、6.09mS/cm、6.59mS/cm、7.09mS/cm、7.59mS/cm、8.09mS/cm、8.59mS/cm、9.09mS/cm、9.59mS/cm、10.09mS/cm、 10.59mS/cm、11.09mS/cm、11.59mS/cm、12.09mS/cm、12.59mS/cm、 13.09mS/cm、13.59mS/cm、14.09mS/cm、14.59mS/cm或约15.09mS/cm，或者电导率可更高或更低。

[0231] 在另一个实例中，液体抗体制剂可以具有用于治疗效力、安全性和储存的任何适合的渗量浓度(osmolality)。例如，液体抗体制剂的渗量浓度可以是约50毫摩尔/千克(mOsm/kg)至约5000mOsm/kg、约50mOsm/kg 至约2000mOsm/kg、约50mOsm/kg至约1000mOsm/kg、约50mOsm/kg 至约750mOsm/kg、或约50mOsm/kg至约500mOsm/kg。在一些实施方案中，液体抗体制剂的渗量浓度可以是约50mOsm/kg、60mOsm/kg、70 mOsm/kg、80mOsm/kg、90mOsm/kg、100mOsm/kg 120mOsm/kg、140 mOsm/kg、160mOsm/kg、180mOsm/kg、200mOsm/kg、

220mOsm/kg、240mOsm/kg、260mOsm/kg、280mOsm/kg、300mOsm/kg、320mOsm/kg、340mOsm/kg、360mOsm/kg、380mOsm/kg、400mOsm/kg、420mOsm/kg、440mOsm/kg、460mOsm/kg、480mOsm/kg、500mOsm/kg、520mOsm/kg、540mOsm/kg、560mOsm/kg、580mOsm/kg、600mOsm/kg、620mOsm/kg、640mOsm/kg、660mOsm/kg、680mOsm/kg、700mOsm/kg、720mOsm/kg、740mOsm/kg、760mOsm/kg、780mOsm/kg、800mOsm/kg、820mOsm/kg、840mOsm/kg、860mOsm/kg、880mOsm/kg、900mOsm/kg、920mOsm/kg、940mOsm/kg、960mOsm/kg、980mOsm/kg、1000mOsm/kg、1050 mOsm/kg、1100mOsm/kg、1150mOsm/kg、1200mOsm/kg、1250mOsm/kg、1300mOsm/kg、1350mOsm/kg、1400mOsm/kg、1450mOsm/kg、约1500 mOsm/kg，或者渗量浓度可更高或更低。

[0232] 包含抗体的脂质体可通过本领域中已知的方法制备，例如Epstein，等，Proc.Natl.Acad.Sci.USA 82:3688(1985)；Hwang，等，Proc.Natl Acad.Sci. USA 77:4030(1980)；以及美国专利No.4,485,045和4,544,545中所述。美国专利No.5,013,556中公开了循环时间延长的脂质体。特别可用的脂质体可通过反相蒸发方法用包含磷脂酰胆碱、胆固醇和PEG衍生化磷脂酰乙醇胺(PEG-PE)的脂质组合物产生。将脂质体通过限定孔径的过滤器挤出以产生具有期望直径的脂质体。

[0233] 还可将活性成分包封在例如通过凝聚技术或通过界面聚合制备的微胶囊中，例如分别为羟甲基纤维素或明胶微胶囊和聚(甲基丙烯酸甲酯)微胶囊；包封在胶体药物递送系统(例如，脂质体、白蛋白微球、微乳剂、纳米颗粒和纳米胶囊)中；或包封在粗滴乳剂(macroemulsion)中。这样的技术在Remington，The Science and Practice of Pharmacy第20版Mack Publishing(2000)中公开。

[0234] 可制备缓释制剂。缓释制剂的合适实例包括含有抗体的固体疏水性聚合物的半透性基质，所述基质是成型制品的形式，例如膜或微胶囊。缓释基质的实例包括聚酯、水凝胶(例如，聚(2-羟乙基-甲基丙烯酸酯)或聚(乙烯醇))、聚丙交酯(美国专利No 3,773,919)、L-谷氨酸与7-乙基-L-谷氨酸酯的共聚物、不可降解的乙烯-乙酸乙烯酯、可降解的乳酸-乙醇酸共聚物如LUPRON DEPOT™(由乳酸-乙醇酸共聚物和醋酸亮丙瑞林构成的可注射微球)、蔗糖乙酸异丁酸酯和聚-D-(-)-3-羟基丁酸。

[0235] 用于体内施用的制剂通常应是无菌的。这容易通过例如经由无菌滤膜过滤来完成。通常将治疗性抗体组合物放置到具有无菌进入口的容器中，例如具有可被皮下注射针刺穿的塞子的静脉内溶液袋或小瓶。

[0236] 根据本发明的组合物可以是用于经口、肠胃外或直肠施用、或通过吸入或吹入施用的单位剂量形式，例如片剂、丸剂、胶囊剂、散剂、颗粒剂、溶液剂或混悬剂或栓剂。在一些情况下，单位剂量形式可供应在可用于将单位剂量施用于对象的预填充容器(例如，预填充注射器)中。

[0237] 对于制备固体组合物(例如片剂)，可将主要活性成分与药用载体(例如，常规压片成分如玉米淀粉、乳糖、蔗糖、山梨糖醇、滑石、硬脂酸、硬脂酸镁、磷酸二钙或树胶)和其他药用稀释剂(例如水)混合以形成含有本发明化合物或其无毒可药用盐的均匀混合物的固体预配制组合物。当将这些预配制组合物称作均匀时，意指活性成分均匀地分散在整个组合物中，使得组合物可容易地再分成等效单位剂量形式，例如片剂、丸剂和胶囊剂。然后，将该固体预配制组合物再分成含有约0.1mg至约500mg本发明活性成分的上述类型的单位剂

量形式。新组合物的片剂或丸剂可被包衣或以其他方式复合以提供提供延长作用的优点的剂型。例如，片剂或丸剂可包含内部剂量和外部剂量组分，后者是在前者上方包膜的形式。这两种组分可通过肠溶层分开，所述肠溶层用于抵抗在胃中的崩解并允许内部组分完整地进入十二指肠或延迟释放。多种材料可以用于这样的肠溶层或包衣，这样的材料包括多种聚合物酸，以及聚合物酸与例如虫胶、鲸蜡醇和醋酸纤维素之材料的混合物。

[0238] 合适的表面活性剂特别地包括非离子试剂，例如聚氧乙烯脱水山梨糖醇(例如TWEEN™ 20、40、60、80或85)和其他脱水山梨糖醇(例如 SPAN™ 20、40、60、80或85)。具有表面活性剂的组合物将常规包含约 0.05％至约5％的表面活性剂，并且可以是约0.1％至约2.5％。应理解的是，如果期望的话，可添加其他成分，例如甘露糖醇或其他可药用载剂。

[0239] 合适的乳剂可使用可商购的脂肪乳剂来制备，例如INTRALIPID™、 LIPOSYN™、INFONUTROL™、LIPOFUNDIN™和LIPIPHYSAN™。活性成分可溶解在预混合的乳剂组合物中，或者作为替代地可将其溶解在油 (例如，大豆油、红花油、棉籽油、芝麻油、玉米油或杏仁油)中，并在与磷脂(例如，卵磷脂、大豆磷脂或大豆卵磷脂)和水混合后形成乳剂。应理解，可添加其他成分，例如甘油或葡萄糖，以调节乳剂的张度 (tonicity)。合适的乳剂通常包含多至20％的油，例如5％至20％。脂肪乳剂可包含约0.1至1.0 1m，特别是约0.1至0.5 1m的脂肪滴，并且具有约pH 5.5至约pH 8.0的pH。

[0240] 乳剂组合物可以是通过将抗体与INTRALIPID™或其组分(大豆油，卵磷脂、甘油和水)混合而制备的那些。

[0241] 用于吸入或吹入的组合物包括在可药用水性或有机溶剂或其混合物中的溶液剂和混悬剂，以及粉末。液体或固体组合物可包含如上所述的合适的可药用赋形剂。在一些实施方案中，组合物通过经口或经鼻呼吸途径施用以用于局部或全身作用。在优选无菌的可药用溶剂中的组合物可通过使用气体来雾化。雾化溶液可直接从雾化装置呼吸，或者雾化装置可连接至面罩、帐篷(tent)或间歇式正压呼吸机。溶液剂、混悬剂或粉末组合物可从以合适方式递送制剂的装置施用，优选经口或经鼻施用。

[0242] 在一些实施方案中，可制备包含本文中所述抗体(例如，调节CGRP 途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体) 的制剂用于任何合适的施用途径，其中抗体量为约0.1mg至约3000mg、约1mg至约1000mg、约100mg至约1000mg、或约100mg至约500mg。在一些情况下，包含本文中所述抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的制剂可包含以下抗体量：最多或至少约0.1mg、1mg、100mg、1mg、10mg、25mg、 50mg、75mg、100mg、125mg、150mg、175mg、200mg、225mg、250 mg、275mg、300mg、325mg、350mg、375mg、400mg、450mg、475 mg、500mg、525mg、550mg、575mg、600mg、625mg、650mg、675 mg、700mg、725mg、750mg、775mg、800mg、825mg、850mg、875 mg、900mg、925mg、950mg、975mg、1000mg、1100mg、1200mg、 1300mg、1400mg、1500mg、1600mg、1700mg、1800mg、1900mg、 2000mg、或约3000mg。

[0243] 在一些实施方案中，可制备包含本文中所述抗体(例如，调节CGRP 途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体) 的液体制剂用于任何合适的施用途径，其中抗体浓度为约0.1mg/mL至约 500mg/mL、约0.1mg/mL至约375mg/mL、约0.1mg/mL至约250mg/mL、约0.1至约175mg/mL、约0.1至100mg/mL、约1mg/mL至约500mg/mL、约1mg/mL至约375mg/mL、约1mg/mL至约300mg/mL、约1mg/mL 至250mg/mL、约1mg/mL至200mg/mL、约1mg/

mL至150mg/mL、约 1mg/mL至约100mg/mL、约10mg/mL至500mg/mL、约10mg/mL至约375mg/mL、约10mg/mL至250mg/mL、约10mg/mL至约150mg/mL、约10mg/mL至100mg/mL、约100mg/mL至500mg/mL、约100mg/mL 至450mg/mL、约100mg/mL至400mg/mL、约100mg/mL至约350 mg/mL、约100mg/mL至约300mg/mL、约100mg/mL至约250mg/mL、 100mg/mL至200mg/mL、或约100mg/mL至约150mg/mL。在一些实施方案中，液体制剂可包含以下浓度的本文中所述的抗体：最多、至少或小于约0.1、0.5、1、5、10，15 20、25、30、35、40、45、50、55、60、65、 70、75、80、85、90、95、100、105110、115、120、125、130、135、140、 145、150、155、160、165、170、175、180、185、190、195、200、210、 220、230、240、250、260、270、280、290、300、310、320、330、340、350、360、370、380、390、400、410、420、430、440、450、460、470、 480、490、或约500mg/mL。

[0244] 抗体制剂可包含一种或更多种组分，其包括抗体和本文中其他部分所述的其他物质。抗体和其他组分针对抗体的治疗效力、安全性和储存可以是任何合适的量和/或任何合适的浓度。在一个实例中，抗体制剂可以是包含以下的溶液：约51.4mg/mL抗体(例如，抗体G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、16至20mM组氨酸、0.1 mg/mL甲硫氨酸、84mg/mL海藻糖二水合物、0.05mg/mL EDTA二钠二水合物和0.2mg/mL聚山梨酯80。

[0245] 在另一个实例中，抗体制剂可包含约200mg/mL抗体(例如，抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、15mM 精氨酸，78mg/mL蔗糖、0.3mg/mL EDTA和0.1mg/mL聚山梨酯80。

[0246] 在另一个实例中，抗体制剂可包含约175mg/mL抗体(例如，抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、20mM 甘氨酸、88mg/mL海藻糖二水合物、0.015mg/mL EDTA和0.25mg/mL 聚山梨酯80。

[0247] 在另一个实例中，抗体制剂可包含约225mg/mL抗体(例如，抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、23mM 天冬酰胺、84mg/mL山梨糖醇、0.1mg/mL EDTA和0.15mg/mL聚山梨酯60。

[0248] 在另一个实例中，抗体制剂可包含约150mg/mL抗体(例如，抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、17mM 天冬酰胺、74mg/mL甘露糖醇、0.025mg/mL EDTA和0.2mg/mL聚山梨酯80。

[0249] 在另一个实例中，抗体制剂可包含约100mg/mL抗体(例如，抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、16mM 精氨酸、87mg/mL甘露糖醇、0.025mg/mL EDTA和0.15mg/mL聚山梨酯20。

[0250] 在另一个实例中，抗体制剂可包含约250mg/mL抗体(例如，抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、25mM 组氨酸、74mg/mL甘露糖醇、0.025mg/mL EDTA和0.25mg/mL聚山梨酯20。

[0251] 在另一个实例中，抗体制剂可包含约50mg/mL抗体(例如，抗体G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、19mM精氨酸、84mg/mL蔗糖、0.05mg/mL EDTA和0.3mg/mL聚山梨酯80。

[0252] 在另一个实例中，抗体制剂可包含约125mg/mL抗体(例如，抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、22mM 甘氨酸、79mg/mL海藻糖二水合物、0.15mg/mL EDTA和0.15mg/mL聚山梨酯80。

[0253] 在另一个实例中，抗体制剂可以是包含以下的溶液：约175mg/mL 抗体(例如，抗体

G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、20mM组氨酸、0.1mg/mL甲硫氨酸、84mg/mL海藻糖二水合物、0.05mg/mL EDTA二钠二水合物和0.2mg/mL聚山梨酯80。

[0254] 在另一个实例中,抗体制剂可包含约200mg/mL抗体(例如,抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、30mM 精氨酸、78mg/mL蔗糖、0.3mg/mL EDTA和0.1mg/mL聚山梨酯80。

[0255] 在另一个实例中,抗体制剂可包含约175mg/mL抗体(例如,抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、20mM 甘氨酸、88mg/mL海藻糖二水合物、0.015mg/mL EDTA和0.15mg/mL 聚山梨酯80。

[0256] 在另一个实例中,抗体制剂可包含约150mg/mL抗体(例如,抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、20mM 组氨酸、84mg/mL蔗糖、0.05mg/mL EDTA和0.2mg/mL聚山梨酯80。

[0257] 在另一个实例中,抗体制剂可包含约225mg/mL抗体(例如,抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、23mM 组氨酸、84mg/mL山梨糖醇、0.1mg/mL EDTA和0.15mg/mL聚山梨酯60。

[0258] 在另一个实例中,抗体制剂可包含约150mg/mL抗体(例如,抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、17mM 天冬酰胺、74mg/mL甘露糖醇、0.3mg/mL EDTA和0.2mg/mL聚山梨酯 80。

[0259] 在另一个实例中,抗体制剂可包含约100mg/mL抗体(例如,抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、16mM 精氨酸、87mg/mL甘露糖醇、0.025mg/mL EDTA和0.25mg/mL聚山梨酯20。

[0260] 在另一个实例中,抗体制剂可包含约250mg/mL抗体(例如,抗体 G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、25mM 组氨酸、89mg/mL甘露糖醇、0.025mg/mL EDTA和0.25mg/mL聚山梨酯20。

[0261] 在另一个实例中,抗体制剂可包含125mg/mL抗体(例如,抗体G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、29mM精氨酸、84mg/mL蔗糖、0.05mg/mL EDTA和0.3mg/mL聚山梨酯80。

[0262] 在另一个实例中,抗体制剂可包含150mg/mL抗体(例如,抗体G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、25mM天冬酰胺、84mg/mL甘露糖醇、0.05mg/mL EDTA和0.2mg/mL聚山梨酯80。

[0263] 在另一个实例中,抗体制剂可包含145mg/mL抗体(例如,抗体G1、其他抗CGRP拮抗剂抗体或调节CGRP途径的单克隆抗体)、22mM组氨酸、72mg/mL海藻糖二水合物、0.05mg/mL EDTA和0.1mg/mL聚山梨酯80。

[0264] 本文中所述的抗体可使用任何合适的方法施用,包括通过注射(例如,静脉内、皮下、腹膜内、肌内等)。如本文中所述,抗体还可通过吸入施用。在一些情况下,可在吸入或不吸入的情况下经鼻施用抗体。通常来说,对于本文中所述的抗体施用,初始候选剂量可以是约2mg/kg。出于本发明的目的,典型的日剂量可以是约3μg/kg至约30μg/kg至约300μg/kg至约3mg/kg至约30mg/kg至约100mg/kg或更多中的任一种,这取决于上文中提及的因素。例如,可使用约1mg/kg、约2.5mg/kg、约5mg/kg、约10mg/kg、约25mg/kg和约30mg/kg的剂量。对于在数天或更长时间内的重复施用,根据病症,持续治疗直至出现期望的症状抑制或直至

达到足够的治疗水平，例如减轻疼痛。示例性的给药方案包括施用约8.5mg/kg 或约10mg/kg的初始剂量，随后是抗体的约2.8mg/kg的维持剂量，或随后是每隔一周约2.8mg/kg的维持剂量。另一个示例性给药方案包括以约 1小时的输注静脉内或皮下向对象每月一次地施用以下剂量：约100mg、 125mg、150mg、200mg、225mg、250mg、275mg、300mg、350mg、 400mg、450mg、500mg、550mg、600mg、约675mg或约900mg。另一个示例性给药方案包括皮下施用约675mg的初始抗体剂量，随后皮下施用约225mg的月抗体剂量进行约2个月，例如约3个月、4个月、5个月、6个月或12个月。另一个给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量，随后每季度以约60分钟的输注静脉内施用约 900mg的剂量持续一年、两年、三年、四年或五年。然而，另一些剂量方案可以是可用的，这取决于从业者希望实现的药动学衰减的模式。例如，在一些实施方案中，考虑每周给药约一次至约四次。这种治疗的过程容易通过常规技术和测定进行监测。给药方案(包括使用的CGRP拮抗剂)可随时间变化。

[0265] 在一些实施方案中，本文中所述并施用于对象的抗体(例如，调节 CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量或量可以是约0.1μg至约3000mg、1mg至1000mg、100mg 至1000mg、100mg至500mg、0.1mg至5000mg、1mg至4000mg、250mg至1000mg、500mg至1000mg、100mg至900mg、400mg至900mg、 10mg至3000mg、10mg至2000mg、100mg至2000mg、150mg至2000 mg、200mg至2000mg、250mg至2000mg、300mg至2000mg、350mg 至2000mg、400mg至2000mg、450mg至2000mg、500mg至2000mg、 550mg至2000mg、600mg至2000mg、650mg至2000mg、700mg至 2000mg、750mg至2000mg、800mg至2000mg、850mg至2000mg、 900mg至2000mg、950mg至2000mg、或1000mg至2000mg。在一些实施方案中，本文中所述并施用于对象的抗体的剂量或量可以是、可以是最多、可以是小于或可以是至少约0.1μg、1μg、100μg、1mg、10mg、 25mg、50mg、75mg、100mg、125mg、150mg、175mg、200mg、225 mg、250mg、275mg、300mg、325mg、350mg、375mg、400mg、450 mg、475mg、500mg、525mg、550mg、575mg、600mg、625mg、650 mg、675mg、700mg、725mg、750mg、775mg、800mg、825mg、850 mg、875mg、900mg、925mg、950mg、975mg、1000mg、1100mg、 1200mg、1300mg、1400mg、1500mg、1600mg、1700mg、1800mg、1900mg、2000mg、或约3000mg。在一些实施方案中，该量为约225mg 至约1000mg，例如约675mg或约900mg。一个示例性给药方案包括皮下施用约675mg的初始抗体剂量，随后皮下施用约225mg的月抗体剂量持续约2个月，例如约3个月、4个月、5个月、6个月或12个月。另一个给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量，随后每季度以约60分钟的输注静脉内施用约900mg的剂量持续一年、两年、三年、四年或五年。然而，另一些剂量方案可以是可用的，这取决于从业者希望实现的药动学衰减的模式。

[0266] 在一些实施方案中，本文中所述并施用于对象的抗体(例如，调节 CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量或量可以是约0.1至500、0.1至100、0.1至50、0.1至20、 0.1至10、1至10、1至7、1至5、或0.1至3mg/kg体重。在一些实施方案中，本文中所述并施用于对象的抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量或量可以是、可以是至多、可以是小于或可以是至少约0.1、0.2、0.3、0.4、 0.5、0.6、0.7、0.8、0.9、1.0、1.5、2.0、2.5、3.0、3.5、4.0、4.5、5.0、 5.5、6.0、6.5、7.0、7.5、8.0、8.5、9.0、9.5、10.0、10.5、11.0、11.5、12.0、 12.5、13.0、13.5、14.0、14.5、15.0、15.5、16.0、16.5、17.0、17.5、18.0、 18.5、19.0、19.5、20、21、22、

23、24、25、26、27、28、29、30、31、 32、33、34、35、36、37、38、39、40、41、42、43、44、45、46、47、48、49、50、55、60、65、70、75、80、85、90、95、100、110、120、 130、140、150、160、170、180、190、200、225、250、275、300、325、 350、375、400、425、450、475或约500mg/kg体重。

[0267] 在一些实施方案中，向对象施用本文中所述抗体(例如，调节CGRP 途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体) 的剂量或量的频率可变化。在一些实施方案中，可在整个治疗过程中向对象给予单次剂量的抗体。在一些实施方案中，向对象施用抗体的剂量或量的频率是恒定的(例如，约每月一次或约每季度一次施用)。在一些实施方案中，向对象施用抗体的剂量或量的频率为约每季度，持续约一年、两年、三年、四年或五年。在一些实施方案中，向对象施用本文中所述抗体的剂量或量的频率是可变的(例如，初始剂量，随后是每月一次的剂量，随后是在约3个月和约7个月时的另外的剂量)。在一些实施方案中，向对象施用抗体的频率为、为至少、为小于或为至多约每天1、2、3、4、5 或6次。在一些实施方案中，向对象施用抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的频率为、为至少、为小于或为至多约每天1、2、3、4、5或6个剂量。

[0268] 在一些实施方案中，向对象施用本文中所述抗体(例如，调节CGRP 途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体) 的剂量或量的频率为、为至少、为小于或为至多每1、2、3、4、5、6、7、 8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、 41、42、43、44、45、46、47、48、49、50、55、60、65、70、75、80、 85、90、95、100、125、150、180或200天约1、2、3、4、5、6、7、8、 9、10、11、12、13、14、15、16、17、18、19或20次。

[0269] 在一些实施方案中，向对象施用本文中所述抗体(例如，调节CGRP 途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体) 的剂量或量的频率为、为至少、为小于或为至多每1、2、3、4、5、6、7、 8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、 41、42、43、44、45、46、47、48、49、50、55、60、65、70、75、80、 85、90、95或100周约1、2、3、4、5、6、7、8、9、10、11、12、13、 14、15、16、17、18、19或20次。在一些实施方案中，向对象施用本文中所述抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的频率为小于或为每周约1、2、3、4、5、 6、7、8、9、10、11、12、13、14或15个剂量。

[0270] 在一些实施方案中，向对象施用抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量或量的频率为、为至少、为小于或为至多每个月、每2个月、每3个月、每4 个月、每5个月、每6个月、每7个月、每8个月、每9个月、每10个月、每11个月、每12个月、每13个月、每14个月、每15个月、每16 个月、每17个月或每18个月约1、2、3、4、5、6、7、8、9、10、11、 12、13、14、15、16、17、18、19或20次。在一些实施方案中，向对象施用抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量或量的频率为每一个月约一次。在一些实施方案中，向对象施用抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量或量的频率为每三个月约一次。在一些实施方案中，向对象施用本文中所述抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP 拮抗剂抗体)的频率小于每个月约1、2、3、4、5、6、7、8、9、10、11、 12、13、14或15个剂量。在一些实施方案中，可每个月向对象施

用(例如,皮下或以输注静脉内)抗体的剂量或量一次、两次、三次、四次、五次、六次、七次、八次、九次、十次或更多次。

[0271] 在一些实施方案中,可每月一次地向对象施用(例如,皮下或以输注静脉内)以下剂量或量的抗体:约50mg、100mg 150mg、200mg、225mg、 250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、 650mg、675mg、700mg、750mg、800mg、850mg、900mg、950mg、 1000mg、1050mg、1100mg、1150mg、1200mg、1250mg、1300mg、 1350mg、1400mg、1450mg、1500mg、1550mg、1600mg、1650mg、 1700mg、1750mg、1800mg、1850mg、1900mg、1950mg、2000mg、2050mg、2100mg、2150mg、2200mg、2250mg、2300mg、2350mg、 2400mg、2450mg、2500mg、2550mg、2600mg、2650mg、2700mg、 2750mg、2800mg、2850mg、2900mg、2950mg、3000mg或更多。在一些实施方案中,可每月一次地向对象施用(例如,皮下或以输注静脉内) 以下剂量或量的抗体:约0.1mg至5000mg、1mg至4000mg、10mg至 3000mg、10mg至2000mg、100mg至2000mg、150mg至2000mg、200 mg至2000mg、250mg至2000mg、300mg至2000mg、350mg至2000 mg、400mg至2000mg、450mg至2000mg、500mg至2000mg、550mg 至2000mg、600mg至2000mg、650mg至2000mg、700mg至2000mg、 750mg至2000mg、800mg至2000mg、850mg至2000mg、900mg至2000mg、950mg至2000mg、或约1000mg至2000mg。在一些实施方案中,每月一次地施用约225mg至约1000mg、例如约225mg的抗体。一个示例性给药方案包括皮下施用约675mg的初始抗体剂量,随后皮下施用约225mg的月抗体剂量持续约2个月,例如约3个月、4个月、5个月、6个月或12个月。另一个给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量,随后每季度以约60分钟的输注静脉内施用约 900mg的剂量持续一年、两年、三年、四年或五年。然而,另一些剂量方案可以是可用的,这取决于从业者希望实现的药动学衰减的模式。

[0272] 在一些实施方案中,可每三个月向对象施用(例如,皮下或以输注静脉内)以下剂量或量的抗体:约50mg、100mg 150mg、200mg、225mg、 250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、 650mg、675mg、700mg、750mg、800mg、850mg、900mg、950mg、1000mg、1050mg、1100mg、1150mg、1200mg、1250mg、1300mg、 1350mg、1400mg、1450mg、1500mg、1550mg、1600mg、1650mg、 1700mg、1750mg、1800mg、1850mg、1900mg、1950mg、2000mg、2050mg、2100mg、2150mg、2200mg、2250mg、2300mg、2350mg、 2400mg、2450mg、2500mg、2550mg、2600mg、2650mg、2700mg、 2750mg、2800mg、2850mg、2900mg、2950mg、3000mg或更多。在一些实施方案中,可每三个月向对象施用(例如,皮下或以输注静脉内) 以下剂量或量的抗体:约0.1mg至5000mg、1mg至4000mg、10mg至 3000mg、10mg至2000mg、100mg至2000mg、150mg至2000mg、200 mg至2000mg、250mg至2000mg、300mg至2000mg、350mg至2000 mg、400mg至2000mg、450mg至2000mg、500mg至2000mg、550mg 至2000mg、600mg至2000mg、650mg至2000mg、700mg至2000mg、 750mg至2000mg、800mg至2000mg、850mg至2000mg、900mg至2000mg、950mg至2000mg、或1000mg至2000mg。在一些实施方案中,每三个月一次地或更少施用约225mg至约1000mg,例如每三个月以输注静脉内施用约900mg。一个示例性给药方案包括皮下施用约675 mg的初始抗体剂量,随后皮下施用约225mg的月抗体剂量持续约2个月,例如约3个月、4个月、5个月、6个月或12个月。另一个给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量,随后每季度以约 60分钟的输注静脉内施用约900mg的剂量持续一年、两年、三年、四年或五年。然而,其他剂量方案可以是可用的,这取决于从业者希望实现的药动学衰减的模式。

[0273] 在一些实施方案中，可每六个月向对象施用(例如，皮下或以输注静脉内)以下剂量或量的抗体：约50mg、100mg、150mg、200mg、225mg、 250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、 650mg、675mg、700mg、750mg、800mg、850mg、900mg、950mg、 1000mg、1050mg、1100mg、1150mg、1200mg、1250mg、1300mg、 1350mg、1400mg、1450mg、1500mg、1550mg、1600mg、1650mg、 1700mg、1750mg、1800mg、1850mg、1900mg、1950mg、2000mg、2050mg、2100mg、2150mg、2200mg、2250mg、2300mg、2350mg、 2400mg、2450mg、2500mg、2550mg、2600mg、2650mg、2700mg、 2750mg、2800mg、2850mg、2900mg、2950mg、3000mg或更多。在一些实施方案中，可每六个月向对象施用(例如，皮下或以输注静脉内) 以下剂量或量的抗体：约0.1mg至5000mg、1mg至4000mg、10mg至3000mg、10mg至2000mg、100mg至2000mg、150mg至2000mg、200 mg至2000mg、250mg至2000mg、300mg至2000mg、350mg至2000 mg、400mg至2000mg、450mg至2000mg、500mg至2000mg、550mg 至2000mg、600mg至2000mg、650mg至2000mg、700mg至2000mg、 750mg至2000mg、800mg至2000mg、850mg至2000mg、900mg至2000mg、950mg至2000mg、或1000mg至2000mg。在一些实施方案中，每6个月一次地或更少施用225mg至1000mg。一个示例性给药方案包括皮下施用约675mg的初始抗体剂量，随后皮下施用约225mg的月抗体剂量持续约2个月，例如约3个月、4个月、5个月、6个月或12个月。另一个给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量，随后每季度以约60分钟的输注静脉内施用约900mg的剂量持续一年、两年、三年、四年或五年。然而，另一些剂量方案可以是可用的，这取决于从业者希望实现的药动学衰减的模式。

[0274] 在一些实施方案中，向对象施用(例如，皮下或静脉内)抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP 拮抗剂抗体)的剂量或量的频率为、为至少、为小于或为至多每季度1、 2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19 或20次。可理解，“季度”可指四分之一年的时间，或者也可指日历季度，例如以下时间：1月1日至3月31日、4月1日至6月30日、7月1日至9月30日、或10月1日至12月31日。在一些情况下，“季度”可指约三个月的时间。

[0275] 在一些实施方案中，可每季度向对象施用(例如，皮下或以输注静脉内)以下剂量或量的抗体：约50mg、100mg、150mg、200mg、225mg、 250mg、300mg、350mg、400mg、450mg、500mg、550mg、600mg、 650mg、675mg、700mg、750mg、800mg、850mg、900mg、950mg、 1000mg、1050mg、1100mg、1150mg、1200mg、1250mg、1300mg、 1350mg、1400mg、1450mg、1500mg、1550mg、1600mg、1650mg、 1700mg、1750mg、1800mg、1850mg、1900mg、1950mg、2000mg、2050mg、2100mg、2150mg、2200mg、2250mg、2300mg、2350mg、 2400mg、2450mg、2500mg、2550mg、2600mg、2650mg、2700mg、 2750mg、2800mg、2850mg、2900mg、2950mg、3000mg或更多。在一些实施方案中，可每季度向对象施用(例如，皮下或以输注静脉内)以下剂量或量的抗体：约0.1mg至5000mg、1mg至4000mg、10mg至3000 mg、10mg至2000mg、100mg至2000mg、150mg至2000mg、200mg 至2000mg、250mg至2000mg、300mg至2000mg、350mg至2000mg、 400mg至2000mg、450mg至2000mg、500mg至2000mg、550mg至 2000mg、600mg至2000mg、650mg至2000mg、700mg至2000mg、 750mg至2000mg、800mg至2000mg、850mg至2000mg、900mg至2000mg、950mg至2000mg、或1000mg至2000mg。一个示例性给药方案包括皮下施用约675mg的初始抗体剂量，随后皮下施用约225mg的月抗体剂量持续约2个月，例如约3个月、4个月、5个月、6个月或12 个月。另一个给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂

量，随后每季度以约60分钟的输注静脉内施用约900mg的剂量持续一年、两年、三年、四年或五年。然而，另一些剂量方案可以是可用的，这取决于从业者希望实现的药动学衰减的模式。

[0276] 在一些实施方案中，施用抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量或量的频率为、为至少、为小于或为至多每年、每两年、每三年、每四年或每五年约1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19 或20次。在一些实施方案中，向对象施用抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的频率小于每年1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、 17、18、19、20、21、22、23、24或25个剂量。

[0277] 在一些实施方案中，可每年一次地向对象施用以下剂量或量的抗体：约50mg、100mg、150mg、200mg、225mg、250mg、300mg、350mg、 400mg、450mg、500mg、550mg、600mg、650mg、675mg、700mg、 750mg、800mg、850mg、900mg、950mg、1000mg、1050mg、1100mg、1150mg、1200mg、1250mg、1300mg、1350mg、1400mg、1450mg、 1500mg、1550mg、1600mg、1650mg、1700mg、1750mg、1800mg、 1850mg、1900mg、1950mg、2000mg、2050mg、2100mg、2150mg、 2200mg、2250mg、2300mg、2350mg、2400mg、2450mg、2500mg、 2550mg、2600mg、2650mg、2700mg、2750mg、2800mg、2850mg、 2900mg、2950mg、3000mg或更多。在一些实施方案中，可每年一次地向对象施用以下剂量或量的抗体：约0.1mg至5000mg、1mg至4000mg、10mg至3000mg、10mg至2000mg、100mg至2000mg、150mg至2000 mg、200mg至2000mg、250mg至2000mg、300mg至2000mg、350mg 至2000mg、400mg至2000mg、450mg至2000mg、500mg至2000mg、 550mg至2000mg、600mg至2000mg、650mg至2000mg、700mg至2000mg、750mg至2000mg、800mg至2000mg、850mg至2000mg、 900mg至2000mg、950mg至2000mg、或1000mg至2000mg。在一些实施方案中，每年一次地或更少施用约450mg至约2000mg。

[0278] 在一些实施方案中，方法可包括在多天向对象施用本文中所述的抗体 (例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗 CGRP拮抗剂抗体)。多天中的2、3、4、5、6、7、8天或更多天可相隔多于1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、 18、19、20、21、22、23、24、25、26、27、28、29、30、35、40、45、 50、55、60、65、70、75天或更多天。在一些实施方案中，多天中的两个可相隔多于1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、 16、17、18、19、20、21、22、23、24、25、26、27、28、29、30天或更多天。此外，在一些实施方案中，在多天中的第一天施用的抗体量可与在第二天施用的抗体量不同(例如，更高或更低)。

[0279] 在一些实施方案中，可向对象施用初始剂量(例如，负荷剂量)的本文中所述抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)，随后以期望的间隔施用一个或更多个另外的剂量。在一些实施方案中，初始剂量和一个或更多个另外的剂量是相同的剂量。在一些实施方案中，所述一个或更多个另外的剂量是与初始剂量不同的剂量。在一些实施方案中，初始剂量和一个或更多个另外的剂量以相同方式施用，即皮下或静脉内施用。在一些实施方案中，所述一个或更多个另外的剂量以与初始剂量不同的方式施用，例如初始剂量可静脉内施用并且所述一个或更多个另外的剂量可皮下施用。在一些实施方案中，施用所述一个或更多个另外的剂量的频率是恒定的(例如，每月或每三个月)。在一些实施方案中，施用所述一个或更多个另外的剂量的频率是可变的(例如，在初始剂量之后一个月施用一个另外的剂量，随后在初始剂量之后三个月再施用其他另外

的剂量)。可使用初始负荷剂量、另外的剂量和另外的剂量的频率(例如,包括本文中所述的那些)的任何期望和/或治疗方案。一个示例性方案包括皮下施用约675mg抗CGRP拮抗剂抗体的初始负荷剂量,随后以一个月的间隔皮下施用约225mg抗体的后续维持剂量。另一个示例性方案包括以约60分钟输注静脉内施用约900 mg抗CGRP拮抗剂抗体的初始剂量,随后以三个月为间隔以约60分钟输注静脉内施用约900mg抗CGRP拮抗剂抗体的后续维持剂量。

[0280] 在一些实施方案中,可向对象施用以下初始剂量的抗体(例如,调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体):约0.1μg、1μg、100μg、1mg、10mg、25mg、50mg、75mg、 100mg、125mg、150mg、175mg、200mg、225mg、250mg、275mg、 300mg、325mg、350mg、375mg、400mg、450mg、475mg、500mg、 525mg、550mg、575mg、600mg、625mg、650mg、675mg、700mg、 725mg、750mg、775mg、800mg、825mg、850mg、875mg、900mg、 925mg、950mg、975mg、1000mg、1500mg、2000mg、或约3000mg;随后施用一个或更多个以下另外剂量的抗体:约0.1μg、1μg、100μg、1 mg、10mg、25mg、50mg、75mg、100mg、125mg、150mg、175mg、 200mg、225mg、250mg、275mg、300mg、325mg、350mg、375mg、 400mg、450mg、475mg、500mg、525mg、550mg、575mg、600mg、 625mg、650mg、675mg、700mg、725mg、750mg、775mg、800mg、 825mg、850mg、875mg、900mg、925mg、950mg、975mg、1000mg、 1500mg、2000mg、或约3000mg。一个示例性方案包括皮下施用约675mg 抗CGRP拮抗剂抗体的初始负荷剂量,随后以一个月的间隔皮下施用约 225mg抗体的后续维持剂量。另一个示例性方案包括以约60分钟输注静脉内施用约900mg抗CGRP拮抗剂抗体的初始剂量,随后以三个月为间隔以约60分钟输注静脉内施用约900mg抗CGRP拮抗剂抗体的后续维持剂量。

[0281] 在一些实施方案中,可将本文中所述的抗体(例如,调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量或量分成亚剂量并以多个亚剂量施用,这取决于例如施用途径和/或施用的特定制剂。例如,在皮下施用剂量的情况下,可将皮下剂量分成多个亚剂量,并且将每个亚剂量施用在不同部位以避免例如在单个部位的较大单次皮下注射。例如,可将900mg的静脉内剂量分成四个各自为225mg 的亚剂量。作为另一个实例,可将675mg的皮下剂量分成三个各自为为 225mg的亚剂量,并且可将每个225mg剂量施用在不同部位,这可帮助最小化在每个部位注射的体积。亚剂量的分配可相等(例如,三个相等的亚剂量)或可不相等(例如,三个亚剂量,其中两个亚剂量是另一个亚剂量的两倍大)。

[0282] 在一些实施方案中,在治疗过程中向对象施用的抗体剂量的数目可不同,这取决于例如在该对象中实现创伤后头痛和/或与创伤后头痛相关的继发症状的发病率降低。例如,在治疗过程中施用的剂量的数目可以是、可以是至少或可以是最多1、2、3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、25、26、27、28、29、 30、31、32、33、34、35、36、37、38、39、40、41、42、43、44、45、 46、47、48、49、50,或者治疗可无限期地给予。在一些情况下,治疗可以是急性的,从而向对象施用最多1、2、3、4、5或6个剂量用于进行治疗。

[0283] 在一些实施方案中,本文中所述抗体(例如,调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量(或亚剂量)或量可配制成液体制剂并且施用(例如,通过皮下注射、通过静脉内注射)于对象。在这样的一些情况下,包含抗体的液体制剂的体积可根据例如液体制剂中的抗体浓度、期望的抗体剂量和/或使用的施用途径而变化。例如,包含本文中所述抗体并且向对象施用(例如,通过注射,例如如皮下注射或静脉

内输注)的液体制剂的体积可以是约0.001mL至约10.0mL、约0.01mL至约5.0mL、约0.1mL至约5mL、约0.1mL至约3mL、约0.5mL至约2.5mL、或约1mL至约2.5mL。例如,包含本文中所述抗体(例如,调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)并且施用(例如,通过注射,例如如皮下注射、或静脉内输注)于对象的液体制剂的体积可以是、可以是至少、可以是小于或可以是最多约0.001、0.005、0.01、0.02、0.03、0.04、0.05、0.06、0.07、0.08、0.09、0.10、0.2、0.3、0.4、0.5、0.6、0.7、0.8、0.9、 1、1.1、1.2、1.3、1.4、1.5、1.6、1.7、1.8、1.9、2.0、2.1、2.2、2.3、2.4、 2.5、2.6、2.7、2.8、2.9、3.0、3.1、3.2、3.3、3.4、3.5、3.6、3.7、3.8、 3.9、4.0、4.1、4.2、4.3、4.4、4.5、4.6、4.7、4.8、4.9、5.0、5.5、6.0、6.5、7.0、7.5、8.0、8.5、9.0、9.5、或约10.0mL。

[0284] 在一些实施方案中,本文中所述抗体(例如,调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量(或亚剂量)或量可在可用于将抗体施用于对象的预填充容器中供应。这样的预填充容器可设计成用于自施用或用于由别人施用。例如,本文中所述抗体的剂量(或亚剂量)或量可作为液体制剂在预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器中提供。在这样的一些实例中,预填充注射器可设计成用于自施用或由别人施用。在一些情况下,预填充注射器或自动注射器可设计成用于皮下施用和/或静脉内施用。

[0285] 出于本发明的目的,抗体的合适剂量可取决于所使用的抗体(或其组合物)、继发症状的类型和严重程度、(持续性)创伤后头痛或其他待治疗病症的类型和严重程度、施用药剂是用于预防目的还是治疗目的、先前治疗、患者的临床病史和针对药剂的响应,以及主治医师的判断。通常来说,临床医师将施用抗体,直至达到实现期望结果的剂量。剂量和/或频率可在治疗过程中变化。

[0286] 例如半衰期的经验考虑通常将有助于确定剂量。例如,可使用与人免疫系统相容的抗体,例如人源化抗体或完全人抗体来延长抗体的半衰期并防止抗体受到宿主免疫系统的攻击。施用频率可在治疗过程中确定和调整,并且通常但不一定基于创伤后头痛或其他病症的治疗和/或抑制和/或改善和/或延迟。或者,抗体的持续连续释放制剂可以是合适的。本领域中已知用于实现缓释的多种制剂和装置。

[0287] 在一个实施方案中,本文中所述抗体(例如,调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的剂量可在已经给予该抗体的一次或更多次施用的个体中凭经验确定。向个体给予增量剂量的抗体。为了评估抗体的效力,可追踪该疾病的指标。

[0288] 根据本发明方法的抗体(例如,调节CGRP途径的单克隆抗体、抗 CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)的施用可以是连续的或间歇的,这取决于例如接受者的生理状况、施用目的是治疗性还是预防性,以及熟练从业人员已知的其他因素。抗体的施用可在预先选定的时间内是基本上连续的,或者可以以一系列间隔的剂量进行,例如在发生(持续性) 创伤后头痛之前、期间或之后;在发生(持续性)创伤后头痛之前;在发生(持续性)创伤后头痛期间;在发生(持续性)创伤后头痛之前和之后;在发生(持续性)创伤后头痛期间和之后;在发生(持续性)创伤后头痛之前和期间;或在发生(持续性)创伤后头痛之前、期间和之后。施用可在任何可能导致(持续性)创伤后头痛的事件之前、期间和/或之后。

[0289] 在一些实施方案中,可存在多于一种抗体。可存在至少一种、至少两种、至少三种、

至少四种、至少五种不同的抗体或更多种抗体。通常来说，这些抗体可具有不彼此产生不利影响的互补活性。本文中所述的抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP 拮抗剂抗体)也可与其他CGRP拮抗剂或CGRP受体拮抗剂联合使用。例如，可使用以下一种或更多种CGRP拮抗剂：针对CGRP的反义分子(包括针对编码CGRP的核酸的反义分子)、CGRP抑制性化合物、CGRP结构类似物、结合CGRP的CGRP受体的显性负突变和抗CGRP受体抗体。抗体还可与用于增强和/或补充药剂有效性的其他药剂联合使用。

[0290] 对创伤后头痛的诊断或评估在本领域中已经充分确立。评估可基于主观量度(例如对症状的患者表征)来进行。在一些实施方案中，如本文中其他部分所述，创伤后头痛的评估可通过头痛小时数进行。例如，创伤后头痛的评估可根据每日头痛小时数、每周头痛小时数、每月头痛小时数和 /或每年头痛小时数。在一些情况下，头痛小时数可由对象报告。

[0291] 治疗效力可通过本领域中公知的方法来评估。例如，可评估疼痛缓解。因此，在一些实施方案中，在施用抗CGRP抗体之后1、2小时或数小时后主观地观察疼痛缓解。在一些实施方案中，在施用抗CGRP抗体之后主观地观察(持续性)创伤后头痛发作的频率。

[0292] 在一些实施方案中，如本文中所述用于在对象中预防、治疗创伤后头痛或降低其发病率的方法可在单次施用本文中所述抗体(例如，调节 CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)之后长时间降低创伤后头痛的发病率。例如，在单次施用之后可降低(持续性)创伤后头痛的发病率持续至少0.5、1、2、3、4、5、6、7、 8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24、 25、26、27、28、29、30、31、32、33、34、35、36、37、38、39、40、 41、42、43、44、45、46、47、48、49、50天或更多天。

[0293] 在一些实施方案中，如本文中所述用于在对象中预防、治疗创伤后头痛或降低其发病率的方法可在向该对象施用一个或更多个剂量的本文中所述抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)之后相对于施用前水平降低对象经历的头痛小时数。例如，相对于对象中的施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每日头痛小时可降低0.5、1、2、3、4、5、6、 7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23 或24个头痛小时。在一些情况下，相对于对象中的施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每日头痛小时可降低0.5％、1％、5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、 55％、60％、65％、70％、75％、80％、85％、90％、95％、99％或更多。在另一个实例中，相对于对象中的施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每周头痛小时可降低0.5、1、5、10、15、 20、25、30、35、40、45、50、55、60、65、70、75个或更多个头痛小时。在一些情况下，相对于对象中的施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每周头痛小时可降低0.5％、1％、5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、60％、65％、70％、 75％、80％、85％、90％、95％、99％或更多。在另一个实例中，相对于施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每月头痛小时可降低0.5、1、5、10、15、20、25、30、35、40、45、50、55、 60、65、70、75、80、85、90、95、100、105、110、115、120、125个或更多个头痛小时。在一些情况下，相对于对象中的施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每周头痛小时可降低0.5％、 1％、5％、10％、15％、20％、25％、30％、35％、40％、45％、50％、55％、 60％、65％、70％、75％、80％、85％、90％、95％、99％或更多。

[0294] 在一些实施方案中，如本文中所述用于在对象中治疗(持续性)创伤后头痛或降低其发病率的方法可在向所述对象施用一个或更多个剂量的本文中所述抗体(例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗CGRP拮抗剂抗体)之后相对于施用前水平降低对象经历的头痛日数。例如，相对于对象中的施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每周头痛日可降低0.5、1、1.5、2、2.5、 3、3.5、4、4.5、5、5.5、6、6.5或7个头痛日。在一些情况下，相对于对象中的施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每周头痛日可降低0.5％、1％、5％、10％、15％、20％、25％、30％、 35％、40％、45％、50％、55％、60％、65％、70％、75％、80％、85％、90％、 95％、99％或更多。在另一个实例中，相对于施用前水平，在向对象施用一个或更多个剂量的抗体之后对象经历的每月头痛日可降低0.5、1、2、3、 4、5、6、7、8、9、10、15、20个或更多个头痛日。

[0295] 在一些实施方案中，方法可包括将一种或更多种另外的药剂与抗体 (例如，调节CGRP途径的单克隆抗体、抗CGRP拮抗剂抗体、单克隆抗 CGRP拮抗剂抗体)同时或先后施用于对象。在一些实施方案中，另外的药剂可以是抗头痛药物，例如本文中其他部分所述的示例性抗头痛药物 (例如，5-HT1激动剂、曲普坦类、麦角生物碱类、阿片类、β-肾上腺素能拮抗剂、NSAID)。在一些实施方案中，与单独使用抗体或一种或更多种另外的药剂相比，治疗效果可更大。因此，可实现抗体与一种或更多种另外的药剂之间的协同效应。在一些实施方案中，所述一种或更多种另外的药剂可由对象预防性摄取。

[0296] B.抗CGRP拮抗剂抗体

[0297] 在一些实施方案中，本发明的方法使用抗体，其可以是抗CGRP拮抗剂抗体。抗CGRP拮抗剂抗体可指阻断、抑制或降低(包括显著地)CGRP 生物活性的任何抗体分子，所述生物活性包括由CGRP信号传导介导的下游途径，例如受体结合和/或针对CGRP的细胞响应的激发。

[0298] 抗CGRP拮抗剂抗体可表现出以下特征中的任意一种或更多种：(a) 与CGRP结合；(b)阻断CGRP与其受体结合；(c)阻断或降低CGRP 受体活化(包括但不限于cAMP活化)；(d)抑制CGRP生物活性或由CGRP 信号传导功能介导的下游途径；(e)预防、改善或治疗创伤后头痛的任何方面；(f)提高CGRP的清除率；以及(g)抑制(降低)CGRP的合成、产生或释放。抗CGRP拮抗剂抗体在本领域中是已知的。参见例如Tan等， Clin.Sci.(Lond).89:565-73，1995；Sigma(Missouri,US)，产品号C7113(克隆#4901)；Plourde等，Peptides 14:1225-1229,1993。

[0299] 在一些实施方案中，抗体以抑制CGRP和/或CGRP途径的方式与 CGRP反应，所述途径包括由CGRP信号传导功能介导的下游途径。在一些实施方案中，抗CGRP拮抗剂抗体识别人CGRP。在一些实施方案中，抗CGRP拮抗剂抗体与人α-CGRP和β-CGRP都结合。在一些实施方案中，抗CGRP拮抗剂抗体结合人和大鼠CGRP。在一些实施方案中，抗CGRP 拮抗剂抗体结合具有CGRP的第25至37位氨基酸的C端片段。在一些实施方案中，抗CGRP拮抗剂抗体结合具有CGRP的第25至37位氨基酸的C端表位。

[0300] 可用于本发明的抗体可涵盖单克隆抗体、多克隆抗体、抗体片段(例如，Fab、Fab′、$F(ab')_2$、Fv、Fc等)、嵌合抗体、双特异性抗体、异缀合物抗体、单链(ScFv)、其突变体、包含抗体部分(例如，结构域抗体) 的融合蛋白、人源化抗体，以及包含具有所需特异性的抗原

识别位点的免疫球蛋白分子的任何其他经修饰构型，包括抗体的糖基化变体、抗体的氨基酸序列变体、以及共价修饰抗体。抗体可以是鼠、大鼠、人或任何其他来源的(包括嵌合抗体或人源化抗体)。

[0301] 在一些实施方案中，抗CGRP拮抗剂抗体是单克隆抗体。在一些实施方案中，抗CGRP拮抗剂抗体是人源化的。在一些实施方案中，抗体是人的。在一些实施方案中，抗CGRP拮抗剂抗体是抗体G1(如本文中所述)。在一些实施方案中，抗CGRP拮抗剂抗体包含抗体G1或在表6中所示 G1变体的一个或更多个CDR(例如一个、两个、三个、四个、五个、或在一些实施方案中，全部六个CDR)。在另一些实施方案中，抗CGRP拮抗剂抗体包含图5中所示的重链可变区的氨基酸序列(SEQ ID NO:1)和图5中所示的轻链可变区的氨基酸序列(SEQ ID NO:2)。

[0302] 在一些实施方案中，抗体包含选自以下的轻链可变区(LCVR)和重链可变区(HCVR)：(a)LCVR17(SEQ ID NO:58)和HCVR22(SEQ ID NO:59)；(b)LCVR18(SEQ ID NO:60)和HCVR23(SEQ ID NO:61)； (c)LCVR19(SEQ ID NO:62)和HCVR24(SEQ ID NO:63)；(d)LCVR20 (SEQ ID NO:64)和HCVR25(SEQ ID NO:65)；(e)LCVR21(SEQ ID NO:66)和HCVR26(SEQ ID NO:67)；(f)LCVR27(SEQ ID NO:68) 和HCVR28(SEQ ID NO:69)；(g)LCVR29(SEQ ID NO:70)和HCVR30 (SEQ ID NO:71)；(h)LCVR31(SEQ ID NO:72)和HCVR32(SEQ ID NO:73)；(i)LCVR33(SEQ ID NO:74)和HCVR34(SEQ ID NO:75)； (j)LCVR35(SEQ ID NO:76)和HCVR36(SEQ ID NO:77)；以及(k) LCVR37(SEQ ID NO:78)和HCVR38(SEQ ID NO:79)。本文中提供了这些区域的序列。US20110305711(SEQ ID NO:5、6、7、12、16、19、24、29、34和39)、US20120294802、US20120294797(SEQ ID NO:51-60) 中描述了抗CGRP抗体的另一些实例，其在此通过引用整体并入。例如，可使用具有以下任何序列的抗体。

[0303] Ab6可变区轻链(人源化)蛋白质序列(US20120294797)

QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASG

[0304] VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIKR (SEQ ID NO:80)

[0305] Ab6轻链(人源化)全长蛋白质序列(US20120294797)

QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASG

[0306] VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:81)

[0307] Ab6可变区重链(人源化)蛋白质序列(US20120294797)

EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGAT

[0308] YYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS (SEQ ID NO:82)

[0309] Ab6重链(人源化)全长蛋白质序列-酵母产生的(US20120294797)

[0310] EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGAT YYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSSAST KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDARVEPKSCDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:83)

[0311] Ab6可变区轻链(人源化)蛋白质序列CDR1(US20120294797)

[0312] QASQSVYHNTYLA (SEQ ID NO:84)

[0313] Ab6可变区轻链(人源化)蛋白质序列CDR2(US20120294797)

[0314] DASTLAS (SEQ ID NO:85)

[0315] Ab6可变区轻链(人源化)蛋白质序列CDR3(US20120294797)

[0316] LGSYDCTNGDCFV (SEQ ID NO:86)

[0317] Ab6可变区重链(人源化)蛋白质序列CDR1(US20120294797)

[0318] GYYMN (SEQ ID NO:87)

[0319] Ab6可变区重链(人源化)蛋白质序列CDR2(US20120294797)

[0320] IGINGATYYASWAKG (SEQ ID NO:88)

[0321] Ab6可变区重链(人源化)蛋白质序列CDR3(US20120294797)

[0322] GDI (SEQ ID NO:89)

[0323] 轻链可变区蛋白质序列CDR3(US20110305711)

[0324] QQGDALPPT (SEQ ID NO:90)

[0325] 轻链可变区蛋白质序列CDR1(US20110305711)

[0326] RASKDISKYL (SEQ ID NO:91)

[0327] 轻链可变区蛋白质序列CDR2(US20110305711)

[0328] YTSGYSH (SEQ ID NO:92)

[0329] 重链可变区蛋白质序列CDR1(US20110305711)

[0330] GYTFGNYWMQ (SEQ ID NO:93)

[0331] 重链可变区蛋白质序列CDR2(US20110305711)

[0332] AIYEGTGKTVYIQKFAD (SEQ ID NO:94)

[0333] 重链可变区蛋白质序列CDR3(US20110305711)

[0334] LSDYVSGFGY (SEQ ID NO:95)

[0335] 轻链可变区蛋白质序列(US20110305711)

[0336] DIQMTQSPSSLSASVGDRVTITCRASKDISKYLNWYQQKPGKAPKLLIYYTSGYHSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDALPPTFGGGTKVEIK (SEQ ID NO:96)

[0337] 重链可变区蛋白质序列(US20110305711)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT

[0338] GKTVYIQKFADRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQGT

TVTVSS (SEQ ID NO:97)

[0339] 轻链蛋白质序列(US20110305711)

DIQMTQSPSSLSASVGDRVTITCRASKDISKYLNWYQQKPGKAPKLLIYYTSGYHSGVP

SRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDALPPTFGGGTKVEIKRTVAAPSVFIF

[0340] PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL

SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:98)

[0341] 重链蛋白质序列(US20110305711)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT

GKTVYIQKFADRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQGT

TVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHT

FPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP

[0342] APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNA

KTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE

PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG

SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG (SEQ ID NO:99)

[0343] 在一些实施方案中,抗体包含经修饰的恒定区,例如本文中所述的免疫惰性恒定区。在一些实施方案中,恒定区如Eur.J.Immunol.(1999) 29:2613-2624;PCT申请No.PCT/GB99/01441;和/或英国专利申请No. 9809951.8中所述进行修饰。在另一些实施方案中,抗体包含含有以下突变的人重链IgG2恒定区:A330P331至S330S331(参考野生型IgG2序列的氨基酸编号)。Eur.J.Immunol.(1999)29:2613-2624。在一些实施方案中,抗体包含含有以下突变的IgG4恒定区:E233F234L235至P233V234A235。在另一些实施方案中,恒定区的N-连接的糖基化为非糖基化的。在一些实施方案中,通过使寡糖附接残基(例如Asn297)和/或为恒定区中N- 糖基化识别序列的一部分的侧翼残基突变来使恒定区的N连接的糖基化为非糖基化的。在一些实施方案中,恒定区的N-连接的糖基化为非糖基化的。可酶促地或通过在糖基化缺陷型宿主细胞中表达来使恒定区的N- 连接的糖基化为非糖基化。

[0344] 抗CGRP拮抗剂抗体与CGRP(例如人α-CGRP)的结合亲和力(K_D) 可以是约0.02至约200nM。在一些实施方案中,结合亲和力为以下中的任一个:约200nM、约100nM、约50nM、约10nM、约1nM、约500pM、约100pM、约60pM、约50pM、约20pM、约15pM、约10pM、约5pM、或约2pM。在一些实施方案中,结合亲和力小于以下中的任一个:约250 nM、约200nM、约100nM、约50nM、约10nM、约1nM、约500pM、约100pM、或约50pM。

[0345] 确定抗体与CGRP的结合亲和力的一种方式是通过测量抗体的单功能Fab片段的结合亲和力。为了获得单功能Fab片段,可用木瓜蛋白酶切割抗体(例如,IgG)或将其重组表达。抗体的抗CGRP Fab片段的亲和力可通过配备有预固定链霉亲和素传感器芯片(SA)的表面等离子体共振(Biacore3000™表面等离子体共振(surface Plasmon resonance,SPR) 系统,Biacore,INC,Piscataway NJ)使用HBS-EP运行缓冲液(0.01M HEPES,pH 7.4,0.15NaCl、3mM EDTA、0.005%v/v表面活性剂P20) 来确定。可将生物素化人CGRP(或任何其他CGRP)稀释到HBS-EP缓冲液中至浓度低于0.5μg/mL,并使用可变的接触时间注射通过单

个芯片通道,以实现两个抗原密度范围:用于详细动力学研究的50至200个响应单位(RU);或用于筛选测定的800至1,000RU。再生研究显示,25% v/v乙醇中的25mM NaOH有效地除去结合的Fab,同时使CGRP的活性在芯片上保持超过200次注射。通常来说,将经纯化Fab样品的连续稀释液(横跨浓度为0.1至10×估计K_D)以100μL/分钟注射1分钟,并允许多至2小时的解离时间。使用已知浓度的Fab(如通过氨基酸分析确定的) 作为标准,通过ELISA和/或SDS-PAGE电泳确定Fab蛋白的浓度。通过使用BIAevaluation程序将数据全局拟合至1:1朗缪尔结合模型(Langmuir binding model)(Karlsson,R.Roos,H.Fagerstam,L.Petersson,B.(1994). Methods Enzymology 6.99-110)来同时获得动力学缔合速率(k_{on})和解离速率(k_{off})。平衡解离常数(K_D)值作为k_{off}/k_{on}计算。该方案适用于确定抗体与任何CGRP的结合亲和力,包括人CGRP、其他哺乳动物(例如小鼠CGRP、大鼠CGRP、灵长类CGRP)的CGRP、以及不同形式的CGRP (例如α和β形式)。一般在25℃下测量抗体的结合亲和力,但也可以在 37℃下测量。

[0346] 包括抗CGRP拮抗剂抗体在内的抗体可通过本领域中已知的任何方法制备。如本文进一步描述的,宿主动物的免疫接种途径和方案通常与用于抗体刺激和产生的已建立的和常规的技术保持一致。用于产生人和小鼠抗体的一般技术是本领域中已知的并且在本文中描述。

[0347] 考虑包括人在内的任何哺乳动物对象或从其来源的抗体产生细胞可被操作以用作产生哺乳动物(包括人)杂交瘤细胞系的基础。通常来说,向宿主动物腹膜内、肌内、经口、皮下、足底内(intraplantar)和/或皮内接种一定量的免疫原(包括如本文中所述的)。

[0348] 杂交瘤可使用Kohler,B.和Milstein,C.(1975)Nature 256:495-497的通用体细胞杂交技术或如Buck,D.W,等,In Vitro,18:377-381(1982)改进的由淋巴细胞和永生化骨髓瘤细胞制备。可在杂交中使用可用的骨髓瘤系,包括但不限于X63-Ag8.653和来自Salk Institute,Cell Distribution Center, San Diego,Calif.,USA的那些。通常来说,该技术涉及使用融合剂(fusogen) (例如聚乙二醇)或通过本领域技术人员公知的电学方式使骨髓瘤细胞与淋巴细胞融合。在融合后,从融合培养基中分离细胞并将其在选择性生长培养基(例如次黄嘌呤-氨基蝶呤-胸苷 (hypoxanthine-aminopterin-thymidine,HAT)培养基)中培养,以消除未杂交的亲本细胞。补充有或不含有血清的本文中所述的任何培养基均可用于培养分泌单克隆抗体的杂交瘤。作为细胞融合技术的另一种替代方案,可使用EBV永生化B细胞来产生本发明的单克隆抗体(例如,单克隆抗 CGRP抗体)。如果期望的话,将杂交瘤扩增并亚克隆,并通过常规免疫测定方法(例如,放射性免疫测定、酶免疫测定或荧光免疫测定)测定上清液的抗免疫原活性。

[0349] 可用作抗体来源的杂交瘤涵盖所有衍生物、产生对CGRP具有特异性的单克隆抗体的亲本杂交瘤的后代细胞、或其一部分。

[0350] 产生这样的抗体的杂交瘤可使用已知的方法在体外或体内培养。如果期望的话,可通过常规的免疫球蛋白纯化方法(例如硫酸铵沉淀、凝胶电泳、透析、色谱和超滤)从培养基或体液分离单克隆抗体。不期望的活性 (如果存在的话)可例如通过以下去除:使制备物经过附着于固相的由免疫原制成的吸附剂,并从免疫原洗脱或释放期望的抗体。用使用双官能剂或衍生化剂与在待免疫接种物种中具有免疫原性的蛋白质(例如,匙孔血蓝蛋白(keyhole limpet hemocyanin)、血清白蛋白、牛甲状腺球蛋白或大豆胰蛋白酶抑制剂)缀

合的人CGRP或含有靶氨基酸序列的片段免疫接种宿主动物可产生抗体(例如,单克隆抗体)的群体,所述双官能剂或衍生化剂例如马来酰亚胺基苯甲酰基磺基琥珀酰亚胺酯(通过半胱氨酸残基缀合)、N-羟基琥珀酰亚胺(通过赖氨酸残基)、戊二醛、琥珀酸酐、SOCl2 或R1N＝C＝NR,其中R和R1是不同的烷基。

[0351] 如果期望的话,可对目的抗体(例如,单克隆或多克隆抗CGRP拮抗剂抗体)进行测序,并随后可将多核苷酸序列克隆到载体中用于表达或增殖。编码目的抗体的序列可保持在宿主细胞的载体中,并随后可扩增宿主细胞并冷冻以供将来使用。在一个替代方案中,多核苷酸序列可被用于遗传操作以使抗体“人源化”或改善抗体的亲和力或其他特征。例如,如果抗体用于人的临床试验和治疗,可将恒定区改造成更类似于人恒定区以避免免疫应答。可需要对抗体序列进行遗传操作以获得对CGRP更大的亲和力和更高的抑制CGRP的效力。对于本领域技术人员显而易见的是,可对抗CGRP拮抗剂抗体产生一个或更多个多核苷酸改变,并仍然保持其对 CGRP的结合能力。

[0352] 使单克隆抗体人源化可包括四个一般性步骤。这些步骤是:(1)确定起始抗体轻链和重链可变结构域的核苷酸和预测氨基酸序列;(2)设计人源化抗体,即决定在人源化过程期间使用哪个抗体框架区;(3)实际人源化方法/技术;以及(4)人源化抗体的转染和表达。参见例如美国专利 No.4,816,567、5,807,715、5,866,692、6,331,415、5,530,101、5,693,761、 5,693,762、5,585,089和6,180,370。

[0353] 已经描述了多种包含来自非人免疫球蛋白的抗原结合位点的“人源化”抗体分子,其包括具有与人恒定结构域融合的啮齿动物或经修饰啮齿动物V区及其相关互补决定区(CDR)的嵌合抗体。参见例如Winter等,Nature 349:293-299(1991);Lobuglio等,Proc.Nat.Acad.Sci.USA 86:4220-4224(1989);Shaw等,J Immunol.138:4534-4538(1987);和Brown 等,Cancer Res.47:3577-3583(1987)。另一些参考文献描述了在与合适的人抗体恒定结构域融合之前接枝到人支持框架区(FR)的啮齿动物CDR。参见,例如,Riechmann等,Nature 332:323-327(1988);Verhoeyen等Science 239:1534-1536(1988);以及Jones等,Nature 321:522-525(1986)。另一篇参考文献描述了由重组修饰的啮齿动物框架区支持的啮齿动物CDR。参见,例如欧洲专利公开No.0519596。这些“人源化”分子被设计成使针对啮齿动物抗人抗体分子的不期望免疫应答最小化,所述不期望免疫应答限制了这些部分在人接受者中的治疗应用的持续时间和有效性。例如,可对抗体恒定区进行改造,使得其具有免疫惰性(例如,不触发补体裂解)。参见,例如PCT公开No.PCT/GB99/01441;英国专利申请No.9809951.8。Daugherty等,Nucl.Acids Res.19:2471-2476(1991)以及美国专利No. 6,180,377、6,054,297、5,997,867、5,866,692、6,210,671和6,350,861;以及PCT公开No.WO 01/27160公开了还可利用的使抗体人源化的另一些方法。

[0354] 在另一个替代方案中,完全人抗体可通过使用已经被改造成表达特定人免疫球蛋白蛋白质的可商购小鼠来获得。设计用于产生更期望的(例如,完全人抗体)或更稳健的免疫应答的转基因动物也可用于产生人源化或人抗体。这样的技术的一些实例是来自Abgenix,Inc.(Fremont,CA)的 XENOMOUSE™以及来自Medarex,Inc.(Princeton,NJ)的HuMAb-Mouse®和TC Mouse™。

[0355] 在一个替代方案中,可使用本领域中已知的任何方法重组制备和表达抗体。在另一种替代方案中,可通过噬菌体展示技术重组制备抗体。参见例如美国专利No.5,565,332、

5,580,717、5,733,743和6,265,150;以及Winter 等,Annu.Rev.Immunol.12:433-455(1994)。或者,噬菌体展示技术 (McCafferty等,Nature 348:552-553(1990))可用于由来自未经免疫接种供体的免疫球蛋白可变(V)结构域基因库体外产生人抗体和抗体片段。根据该技术,将抗体V结构域基因框内克隆到丝状噬菌体(例如M13或 fd)的主要或次要外壳蛋白基因中,并在噬菌体颗粒的表面上作为功能性抗体片段展示。由于丝状颗粒含有噬菌体基因组的单链DNA拷贝,因此基于抗体的功能特性的选择也导致选择出编码表现出这些特性的抗体的基因。因此,噬菌体模拟B细胞的一些特性。噬菌体展示可以以多种形式进行;综述参见例如Johnson,Kevin S.和Chiswell,David J.,Current Opinion in Structural Biology 3:564-571(1993)。V基因片段的数种来源可用于噬菌体展示。Clackson等,Nature 352:624-628(1991)从来自经免疫接种小鼠的脾的V基因的小随机组合文库中分离出一系列多样性抗噁唑酮抗体。可构建来自未经免疫接种人供体的V基因库,并基本上按照Mark等,J.Mol. Biol.222:581-597(1991)或Griffith等,EMBO J.12:725-734(1993)所述的技术分离针对一系列多样性抗原(包括自身抗原)的抗体。在天然免疫应答中,抗体基因以高速率积累突变(体细胞高频突变)。引入的一些变化将赋予更高的亲和力,并且展示高亲和力表面免疫球蛋白的B细胞在随后的抗原攻击期间优先复制并分化。这种自然过程可通过采用称为“链混编 (chain shuffling)”的技术来模拟。Marks,等,Bio/Technol.10:779-783 (1992))。在该方法中,可如下改善通过噬菌体展示获得的“初级”人抗体的亲和力:用从未经免疫接种供体获得的V结构域基因的天然变体(库)先后替换重链和轻链V区基因。该技术允许产生亲和力在pM-nM范围内的抗体和抗体片段。Waterhouse等,Nucl.Acids Res.21:2265-2266(1993)已经描述了用于制备非常大噬菌体抗体库(也称为“一切根源文库 (mother-of-all library)”)的策略。基因改组也可用于由啮齿动物抗体获得人抗体,其中人抗体对起始啮齿动物抗体具有类似的亲和力和特异性。根据这种也称为“表位印记”的方法,将通过噬菌体展示技术获得的啮齿动物抗体的重链或轻链V结构域基因替换为人V结构域基因库,从而产生啮齿动物-人嵌合体。对抗原的选择导致分离能够恢复功能性抗原结合位点的人可变区,即表位控制(印记)配偶体的选择。当重复该过程以替换剩余的啮齿动物V结构域时,获得人抗体(参见PCT公开No.WO 93/06213,公开于1993年4月1日)。与通过CDR接枝的传统啮齿动物抗体人源化不同,该技术提供没有啮齿动物来源的框架残基或CDR残基的完全人抗体。

[0356] 明显的是,尽管上述讨论涉及人源化抗体,但所讨论的一般性原理适用于定制用于例如狗、猫、灵长类、马和牛的抗体。还明显的是,本文中所述的使抗体人源化的一个或更多个方面可组合,例如CDR接枝、框架突变和CDR突变。

[0357] 抗体可通过以下重组制备:首先从宿主动物中分离抗体和抗体产生细胞,获得基因序列,并使用基因序列在宿主细胞(例如,CHO细胞)中重组表达抗体。可采用的另一种方法是在植物(例如,烟草)或转基因乳中表达抗体序列。已经公开了在植物或乳中重组表达抗体的方法。参见例如Peeters,等Vaccine 19:2756(2001);Lonberg,N.和D.Huszar Int.Rev. Immunol 13:65(1995);以及Pollock,等,J Immunol Methods 231:147(1999)。用于制备抗体衍生物(例如,人源化的、单链的等)的方法是本领域中已知的。

[0358] 还可采用免疫测定和流式细胞术分选技术(例如荧光激活细胞分选 (FACS))来分离对CGRP具有特异性的抗体。

[0359] 抗体可与多种不同的载体结合。载体可以是活性的和/或惰性的。公知的载体的实例包括聚丙烯、聚苯乙烯、聚乙烯、右旋糖酐、尼龙、淀粉酶、玻璃、天然和改性纤维素、聚丙烯酰胺、琼脂糖和磁铁矿。载体的性质可以是可溶性或不溶性的。本领域技术人员将知晓其他合适的用于结合抗体的载体，或将能够使用常规实验来确定这样的载体。在一些实施方案中，载体包含靶向心肌的部分。

[0360] 编码单克隆抗体的DNA可容易地使用常规方法来分离和测序(例如，通过使用能够与编码单克隆抗体的重链和轻链的基因特异性结合的寡核苷酸探针)。杂交瘤细胞充当这样的DNA的优选来源。一旦分离，可将DNA置于表达载体(例如PCT公开No.WO 87/04462中公开的表达载体) 中，然后将其转染到不另外产生免疫球蛋白蛋白质的宿主细胞(例如大肠杆菌(E.coli)细胞、猿猴COS细胞、中国仓鼠卵巢(CHO)细胞或骨髓瘤细胞)中，以在重组宿主细胞中获得单克隆抗体的合成。参见例如PCT 公开No.WO 87/04462。例如，也可通过以下修饰DNA：替换人重链和轻链恒定结构域的编码序列以替代同源鼠序列，Morrison等，Proc.Nat.Acad. Sci.81:6851(1984)；或者使非免疫球蛋白多肽的编码序列的全部或一部分与免疫球蛋白编码序列共价连接。以此方式，制备具有本文中抗CGRP单克隆抗体的结合特异性的“嵌合”或“杂合”抗体。

[0361] 抗体(例如，抗CGRP拮抗剂抗体)和来自抗体的多肽可使用本领域中已知的方法鉴定或表征，从而检测和/或测量CGRP生物活性的降低、提高或中和。例如，也可通过以下鉴定抗CGRP拮抗剂抗体：将候选试剂与CGRP一起孵育并监测以下特征中的任意一种或更多种：(a)与CGRP 结合；(b)阻断CGRP与其受体结合；(c)阻断或降低CGRP受体活化(包括cAMP活化)；(d)抑制CGRP生物活性或由CGRP信号传导功能介导的下游途径；(e)预防、改善或治疗创伤后头痛的任何方面；(f)提高CGRP 的清除率；以及(g)抑制(降低)CGRP的合成、产生或释放。在一些实施方案中，可通过以下鉴定抗CGRP拮抗剂抗体或多肽：将候选药剂与 CGRP一起孵育并监测CGRP的结合和/或其生物活性的伴随降低或中和。结合测定可用经纯化的CGRP多肽进行，或用天然表达或转染以表达 CGRP多肽的细胞进行。在一个实施方案中，结合测定是竞争性结合测定，其中评价候选抗体与已知抗CGRP拮抗剂竞争CGRP结合的能力。该测定可以以多种形式进行，包括ELISA形式。在另一些实施方案中，通过以下鉴定抗CGRP拮抗剂抗体：将候选试剂与CGRP一起孵育，并监测在细胞表面上表达的CGRP受体的结合及其活化的伴随抑制。在一些实施方案中，抗CGRP受体抗体可用于本文中所述的任何方法。例如，可使用如 US20100172895和美国专利No.9,102,731中所述的抗CGRP受体抗体，其在此通过引用整体并入。

[0362] 在初步鉴定后，可通过已知用于测试靶向生物活性的生物测定来进一步确定和改善候选抗体(例如，抗CGRP拮抗剂抗体)的活性。或者，生物测定可用于直接筛选候选物。例如，CGRP促进响应性细胞中的许多可测量变化。这些包括但不限于刺激细胞(例如，SK-N-MC细胞)中的cAMP。还可使用动物模型来测量拮抗剂活性，例如测量由刺激大鼠隐神经诱导的皮肤血管扩张。Escott等，Br.J.Pharmacol.110:772-776,1993。创伤后头痛的动物模型还可以用于测试拮抗剂抗体或多肽的效力。Reuter，等， Functional Neurology(15)增刊3，2000。在实施例中详细描述了用于鉴定和表征抗CGRP拮抗剂抗体或多肽的一些方法。

[0363] 包括抗CGRP拮抗剂抗体在内的抗体可使用本领域中公知的方法表征。例如，一种方法是鉴定与其结合的表位或“表位作图”。本领域中已知有许多方法用于对蛋白质上表位

的位置进行作图和表征,包括解析抗体- 抗原复合物的晶体结构、竞争测定、基因片段表达测定和基于合成肽的测定,如例如在Harlow和Lane,Using Antibodies,a Laboratory Manual,Cold Spring Harbor Laboratory Press,Cold Spring Harbor,New York,1999的第11 章中所述。在另一个实例中,表位作图可用于确定与抗CGRP拮抗剂抗体结合的序列。表位作图可从多种来源商购获得,例如Pepscan Systems (Edelhertweg 15,8219 PH Lelystad,The Netherlands)。表位可以是线性表位,即包含在单个氨基酸区段中,或可以是通过氨基酸的三维相互作用形成的构象表位,其可不一定包含在单个区段中。可分离或合成(例如,重组地)不同长度(例如,至少4至6个氨基酸长)的肽,并将其用于与抗 CGRP拮抗剂抗体的结合测定。在另一个实例中,与抗CGRP拮抗剂抗体结合的表位可通过以下在系统性筛选中确定:使用来自CGRP序列的重叠肽,并确定抗CGRP拮抗剂抗体的结合。根据基因片段表达测定,将编码 CGRP的开放阅读框随机或通过特定的遗传构建片段化,并确定CGRP的表达片段与受试抗体的反应性。例如,基因片段可通过PCR产生,并随后转录并在存在放射性氨基酸的情况下在体外翻译成蛋白质。然后通过免疫沉淀和凝胶电泳来确定抗体与放射性标记的CGRP片段的结合。某些表位还可通过使用展示在噬菌体颗粒表面上的大随机肽序列文库(噬菌体文库)来鉴定。或者,可在简单结合测定中测试确定的重叠肽片段文库与受试抗体的结合。在另一个实例中,可进行抗原结合结构域的诱变、结构域交换实验和丙氨酸扫描诱变来鉴定表位结合所需、足够和/或必需的残基。例如,可使用突变体CGRP来进行结构域交换实验,在突变体CGRP中 CGRP多肽的不同片段已被来自密切相关但抗原性不同的蛋白质(例如神经营养因子(neurotrophin)蛋白质家族的另一成员)的序列取代(交换)。通过评估抗体与突变体CGRP的结合,可评估特定CGRP片段对抗体结合的重要性。

[0364] 可用于表征包括抗CGRP拮抗剂抗体在内的抗体的另一种方法是使用与已知与相同抗原(即CGRP上的多种片段)结合的其他抗体的竞争测定来确定抗CGRP拮抗剂抗体与其他抗体是否与相同的表位结合。竞争测定对于本领域技术人员来说是公知的。

[0365] 表达载体可用于指导抗体(包括抗CGRP拮抗剂抗体)的表达。本领域技术人员熟悉施用表达载体以在体内获得外源蛋白的表达。参见例如美国专利No.6,436,908、6,413,942和6,376,471。表达载体的施用包括局部或全身施用,包括注射、经口施用、粒子枪(particle gun)或插管施用 (catheterized administration),以及表面施用。在另一个实施方案中,将表达载体直接施用于交感神经干或神经节,或者施用到冠状动脉、心房、心室或心包膜中。

[0366] 还可使用含有表达载体或亚基因组多核苷酸的治疗性组合物的靶向递送。受体介导的DNA递送技术描述于例如Findeis等,Trends Biotechnol. (1993)11:202;Chiou等,Gene Therapeutics:Methods and Applications of Direct Gene Transfer(J.A.Wolff,编辑)(1994);Wu等,J.Biol.Chem.(1988) 263:621;Wu等,J.Biol.Chem.(1994)269:542;Zenke等,Proc.Natl.Acad. Sci.USA(1990)87:3655;Wu等,J.Biol.Chem.(1991)266:338中。在基因治疗方案中,包含多核苷酸的治疗性组合物以约100ng至约200mg DNA 施用以用于局部施用。在基因治疗方案期间,也可使用约500ng至约50 mg、约1μg至约2mg、约5μg至约500μg、以及约20μg至约100μg DNA 的浓度范围。治疗性多核苷酸和多肽可使用基因递送载剂来递送。基因递送载剂可以是病毒或非病毒来源的(一般参见Jolly,Cancer Gene Therapy (1994)1:51;Kimura,Human Gene Therapy(1994)5:845;Connelly,Human Gene

Therapy(1995)1:185;和Kaplitt,Nature Genetics(1994)6:148)。这样的编码序列的表达可使用内源哺乳动物启动子或异源启动子来诱导。编码序列的表达可以是组成型的或调节型的。

[0367] 用于递送期望多核苷酸并在期望细胞中表达的基于病毒的载体在本领域中是公知的。示例性的基于病毒的载剂包括但不限于重组逆转录病毒 (参见例如PCT公开No.WO 90/07936、WO 94/03622、WO 93/25698、 WO 93/25234、WO 93/11230、WO 93/10218、WO 91/02805;美国专利No. 5,219,740和4,777,127;英国专利No.2,200,651;以及欧洲专利No.0 345 242);基于甲病毒(alphavirus)的载体(例如,辛德毕斯病毒载体(Sindbis virus vector)、塞姆利基森林病毒(Semliki forest virus)(ATCC VR-67; ATCC VR-1247)、罗斯河病毒(Ross River virus)(ATCC VR-373;ATCC VR-1246)和委内瑞拉马脑炎病毒(Venezuelan equine encephalitis virus) (ATCC VR-923;ATCC VR-1250;ATCC VR 1249;ATCC VR-532))以及腺相关病毒(adeno-associated virus,AAV)载体(参见,例如PCT公开No.WO 94/12649、WO 93/03769、WO 93/19191、WO 94/28938、WO 95/11984和WO 95/00655))。还可采用与经杀伤腺病毒连接的DNA的施用,如Curiel,Hum.Gene Ther.(1992)3:147中所述。

[0368] 还可采用非病毒递送载剂和方法,包括但不限于与单独的死腺病毒连接或未连接的聚阳离子缩合DNA(参见例如Curiel,Hum.Gene Ther.(1992) 3:147);配体连接的DNA(参见例如Wu,J.Biol.Chem.(1989)264:16985);真核细胞递送载剂细胞(参见例如美国专利No.5,814,482;PCT公开No. WO 95/07994、WO 96/17072、WO 95/30763和WO 97/42338)以及核酸电荷中和或与细胞膜融合。也可使用裸DNA。示例性的裸DNA引入方法在PCT公开No.WO 90/11092和美国专利No.5,580,859中描述。可充当基因递送载剂的脂质体描述于美国专利No.5,422,120;PCT公开No.WO 95/13796、WO 94/23697、WO 91/14445,以及EP 0524968中。另外的方法在Philip,Mol.Cell Biol.(1994)14:2411和Woffendin,Proc.Natl.Acad.Sci.(1994)91:1581中描述。

[0369] C.抗体G1以及相关抗体、多肽、多核苷酸、载体和宿主细胞

[0370] 本发明涵盖组合物,包括药物组合物,其包含抗体G1及其在表6中所示变体或者来自于抗体G1的多肽及其在表6中所示变体;以及包含编码G1及其变体或所述多肽之序列的多核苷酸。在一些实施方案中,组合物包含与CGRP结合的一种或更多种抗体或多肽(其可以是或可以不是抗体),和/或包含编码与CGRP结合的一种或更多种抗体或多肽的序列的一种或更多种多核苷酸。这些组合物还可包含本领域中公知的合适的赋形剂,例如可药用赋形剂,包括缓冲剂。

[0371] 在一些实施方案中,本发明的抗CGRP拮抗剂抗体和多肽的特征在于以下特征中的任何(一个或更多个):(a)与CGRP结合;(b)阻断CGRP 与其受体的结合;(c)阻断或降低CGRP受体活化(包括cAMP活化); (d)抑制CGRP生物学活性或由CGRP信号传导功能介导的下游途径; (e)预防、改善或治疗创伤后头痛的任何方面;(f)提高CGRP的清除;以及(g)抑制(降低)CGRP合成、产生或释放。

[0372] 在一些实施方案中,本发明提供了以下中的任何或者包含以下任一种的组合物(包括药物组合物):(a)抗体G1或其在表6中所示变体;(b) 抗体G1或其在表6中所示变体的片段或区域;(c)抗体G1或其在表6 中所示变体的轻链;(d)抗体G1或其在表6中所示变体的

重链;(e)来自抗体G1或其在表6中所示变体的轻链和/或重链的一个或更多个可变区;(f)抗体G1或其在表6中所示变体的一个或更多个CDR(一个、两个、三个、四个、五个或六个CDR);(g)来自抗体G1的重链的CDR H3; (h)来自抗体G1或其在表6中所示变体的轻链的CDR L3;(i)来自抗体G1或其在表6中所示变体的轻链的三个CDR;(j)来自抗体G1或其在表6中所示变体的重链的三个CDR;(k)来自抗体G1或其在表6中所示变体的轻链的三个CDR和重链的三个CDR;以及(l)包含(b)至(k) 中任一个的抗体。在一些实施方案中,本发明还提供包含上述任何一种或更多种的多肽。

[0373] 图5中图示地描绘了抗体G1的CDR部分(包括Chothia和Kabat CDR)。CDR区的确定完全在本领域技术的范围内。应理解的是,在一些实施方案中,CDR可以是Kabat CDR和Chothia CDR的组合(也称为"组合的CDR"或"延伸的CDR")。在一些实施方案中,CDR是Kabat CDR。在另一些实施方案中,CDR是Chothia CDR。换句话说,在具有多于一个 CDR的一些实施方案中,CDR可以是以下任一种:Kabat CDR、Chothia CDR、组合的CDR、或其组合。

[0374] 在一些实施方案中,本发明提供了多肽(其可以是或可以不是抗体),其包含与G1或其在表6中所示变体的至少一个CDR、至少两个、至少三个、至少四个、至少五个或全部六个CDR基本同一的至少一个CDR、至少两个、至少三个、或至少四个、至少五个或全部六个CDR。另一些实施方案包括抗体,其具有与G1的或来自于G1的至少两个、三个、四个、五个或六个CDR基本同一的至少两个、三个、四个、五个或六个CDR。在一些实施方案中,至少一个、两个、三个、四个、五个或六个CDR与和G1或其在表6中所示变体的至少一个、两个、三个、四个、五个或六个CDR至少约85%、86%、87%、88%、89%、90%、95%、96%、97%、 98%、或99%同一。应理解,为了本发明的目的,一般保留了结合特异性和/或整体活性,尽管活性程度与G1或其在表6中所示变体相比可变化(可更大或更小)。

[0375] 在一些实施方案中,本发明还提供了包含G1或其在表6中所示变体的氨基酸序列的多肽(其可以是或可以不是抗体),其具有以下任一种: G1或其在表6中所示变体的至少5个连续氨基酸、至少8个连续氨基酸、至少约10个连续氨基酸、至少约15个连续氨基酸、至少约20个连续氨基酸、至少约25个连续氨基酸、至少约30个连续氨基酸,其中至少3个氨基酸来自G1(图5)或其在表6中所示变体的可变区。在一个实施方案中,可变区来自G1的轻链。在另一个实施方案中,可变区来自G1的重链。示例性多肽具有来自G1的重链可变区和轻链可变区二者的连续氨基酸(上述长度)。在另一个实施方案中,5个(或更多个)连续氨基酸来自图5中所示G1的互补决定区(CDR)。在一些实施方案中,连续氨基酸来自G1的可变区。

[0376] 抗CGRP拮抗剂抗体和多肽与CGRP(例如人α-CGRP)的结合亲和力(K_D)可以是约0.06至约200nM。在一些实施方案中,结合亲和力为约200nM、100nM、约50nM、约10nM、约1nM、约500pM、约100 pM、约60pM、约50pM、约20pM、约15pM、约10pM、约5pM、或约2pM中的任一个。在一些实施方案中,结合亲和力小于约250nM、约 200nM、约100nM、约50nM、约10nM、约1nM、约500pM、约100 pM、或约50pM中的任一个。

[0377] 在一些实施方案中,本发明还提供了制备这些抗体或多肽中的任一种的方法。本发明的抗体可通过本领域中已知的方法制备。多肽可通过抗体的蛋白水解或其他降解、通过上述重组方法(即,单一多肽或融合多肽) 或通过化学合成产生。抗体的多肽(尤其是至多约50个氨基酸的较短多肽)通过化学合成方便地制备。化学合成的方法在本领域中是已知的并且可商购。例如,抗体可使用固相方法通过自动化多肽合成仪产生。也参见美国专利

No.5,807,715、4,816,567和6,331,415。

[0378] 在另一个替代方案中，抗体可使用本领域中公知的方法重组制备。在一个实施方案中，多核苷酸包含编码SEQ ID NO:9和SEQ ID NO:10中所示抗体G1的重链和/或轻链可变区的序列。在另一个实施方案中，将包含SEQ ID NO:9和SEQ ID NO:10中所示核苷酸序列的多核苷酸克隆到一个或更多个载体中用于表达或扩增。编码目的抗体的序列可保持在宿主细胞中的载体中，并随后可扩增并冷冻宿主细胞以备将来使用。本文中进一步描述了载体(包括表达载体)和宿主细胞。

[0379] 在一些实施方案中，本发明还涵盖本发明抗体(例如G1)的单链可变区片段(“scFv”)。单链可变区片段通过使用短连接肽连接轻链和/或重链可变区来制备。Bird等(1988)Science 242:423-426。连接肽的一个实例是(GGGGS)3(SEQ ID NO:57)，其在一个可变区的羧基末端与另一个可变区的氨基末端之间桥接约3.5nm。已设计和使用了其他序列的接头。Bird 等(1988)。接头可进行修饰以实现另外的功能，例如附接药物的或附接于固体支持物。单链变体可重组产生或合成产生。对于scFv的合成产生，可使用自动化合成仪。对于scFv的重组产生，可将含有编码scFv的多核苷酸的合适质粒导入合适的宿主细胞、真核细胞(例如酵母、植物、昆虫或哺乳动物细胞)或原核细胞(例如大肠杆菌)。编码目的scFv的多核苷酸可通过常规操作(例如多核苷酸的连接)来制备。所得scFv可使用本领域中已知的标准蛋白质纯化技术来分离。

[0380] 还涵盖了单链抗体的其他形式，例如双抗体。双抗体是二价、双特异性抗体，其中VH和VL结构域在单个多肽链上表达，但使用的接头太短而允许同一链上的两个结构域之间配对，从而迫使结构域与另一链的互补结构域配对并产生两个抗原结合位点(参见例如Holliger,P.,等(1993)Proc. Natl.Acad Sci.USA 90:6444-6448;Poljak,R.J.,等(1994)Structure 2:1121-1123)。

[0381] 例如，可使用本文中公开的抗体制备对至少两种不同抗原具有结合特异性的双特异性抗体、单克隆抗体。制备双特异性抗体的方法是本领域中已知的(参见例如Suresh等，1986,Methods in Enzymology 121:210)。传统上，双特异性抗体的重组产生基于两个免疫球蛋白重链-轻链对的共表达，其中两个重链具有不同的特异性(Millstein和Cuello，1983,Nature 305,537-539)。

[0382] 根据制备双特异性抗体的一种方法，将具有期望结合特异性(抗体- 抗原组合位点)的抗体可变结构域与免疫球蛋白恒定结构域序列融合。所述融合优选是与免疫球蛋白重链恒定结构域(包含铰链区、CH2区和CH3 区的至少一部分)。优选地，包含轻链结合所必需位点的第一重链恒定区 (CH1)存在于至少一个融合物中。编码免疫球蛋白重链融合物和(必要时)免疫球蛋白轻链的DNA插入到分开的表达载体中，并共转染到合适的宿主生物中。当在构建中使用的不等比例的三种多肽链提供最佳产量时，这为调节实施方式中三种多肽片段的相互比例提供了极大的灵活性。然而，当至少两个多肽链以相同比例表达时导致高产率或者当比例没有特别意义时，可将两个或全部三个多肽链的编码序列插入一个表达载体中。

[0383] 在一种方法中，双特异性抗体由一个臂中具有第一结合特异性的杂合免疫球蛋白重链和另一个臂中杂合免疫球蛋白重链-轻链对(提供第二结合特异性)构成。这种仅在双特异性分子的一半中具有免疫球蛋白轻链的不对称结构有利于将期望的双特异性化合物

与不期望的免疫球蛋白链组合分开。该方法在1994年3月3日公开的PCT公开No.WO 94/04690中描述。

[0384] 包含两个共价连接的抗体的异缀合抗体(Heteroconjugate antibody) 也在本发明的范围内。这种抗体已用于将免疫系统细胞靶向不需要的细胞 (美国专利号4,676,980),以及用于治疗HIV感染(PCT申请公开No.WO 91/00360和WO 92/200373;EP 03089)。可使用任何方便的交联方法制备异缀合抗体。合适的交联剂和技术是本领域中公知的,并且在美国专利 No.4,676,980中有所描述。

[0385] 嵌合或杂合抗体也可使用已知的合成蛋白质化学方法(包括涉及交联剂的那些)在体外制备。例如,可使用二硫化物交换反应或通过形成硫醚键来构建免疫毒素。用于该目的的合适试剂的实例包括亚氨基硫醇盐 (iminothiolate)和4-巯基丁酰亚胺酸甲酯(methyl-4-mercaptobutyrimidate)。

[0386] 可使用本领域中已知的任何方法制备包含抗体G1或其在表6中所示变体的一个或更多个CDR或者来自于抗体G1或其在表6中所示变体的一个或更多个CDR的人源化抗体。例如,可使用四个一般步骤来使单克隆抗体人源化。

[0387] 在一些实施方案中,本发明涵盖对抗体G1或其在表6中所示变体的修饰,包括不显著影响其性质的功能等同抗体和具有增强或降低的活性和 /或亲和力的变体。例如,抗体G1或其在表6中所示变体的氨基酸序列可被突变以获得对CGRP具有期望结合亲和力的抗体。多肽的修饰是本领域中的常规实践,并且不需要在本文中详细描述。实施例中举例说明了多肽的修饰。经修饰多肽的实例包括具有氨基酸残基的保守替换、一个或更多个不显著有害地改变功能活性的氨基酸缺失或添加、或者使用化学类似物的多肽。

[0388] 氨基酸序列插入包括长度为一个残基至含有数百个或更多个残基的多肽的氨基末端和/或羧基末端融合,以及单个或多个氨基酸残基的序列内插入。末端插入的实例包括具有N-末端甲硫氨酰残基的抗体或与表位标签融合的抗体。抗体分子的其他插入变体包括延长抗体的血清半衰期的酶或多肽与抗体的N-末端或C-末端的融合。

[0389] 替换变体为抗体分子中移除至少有一个氨基酸残基,并在其位置插入不同的残基。替换诱变最感兴趣的位点包括高变区,但也考虑了FR改变。表1中在“保守替换”标题下示出了保守替换。如果这种替换导致生物活性发生变化,则可引入表1中称为“示例性替换”或下文中关于氨基酸类别进一步描述的更实质性变化,并筛选产物。

[0390] 表1.氨基酸替换

[0391]

原始残基	保守替换	示例性替换
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; 正亮氨酸
Leu (L)	Ile	正亮氨酸; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; 正亮氨酸

[0392] 通过选择在维持以下的作用方面显著不同的替换来实现抗体的生物学性质的实质性修饰：(a)替换区域中多肽骨架的结构，例如作为片层或螺旋构象，(b)分子在靶位点处的电荷或疏水性，或(c)侧链的体积(bulk)。根据常见的侧链性质将天然存在的残基分成几组：

[0393] (1)非极性：正亮氨酸、Met、Ala、Val、Leu、Ile；

[0394] (2)极性无电荷：Cys、Ser、Thr、Asn、Gln；

[0395] (3)酸性(带负电荷)：Asp、Glu；

[0396] (4)碱性(带正电荷)：Lys、Arg；

[0397] (5)影响链取向的残基：Gly、Pro；以及

[0398] (6)芳香族：Trp、Tyr、Phe、His。

[0399] 通过将这些类别中一个类别的成员替换为另一个类别的来进行非保守性替换。

[0400] 任何不参与维持抗体的适当构象的半胱氨酸残基也可通常用丝氨酸替换，以改善分子的氧化稳定性并防止异常交联。相反，可将半胱氨酸键添加至抗体以改善其稳定性，特别是当抗体是抗体片段(例如Fv片段) 时。

[0401] 氨基酸修饰的范围可以从改变或修饰一个或更多个氨基酸到区域(例如可变区)

的完全重新设计。可变区的变化可改变结合亲和力和/或特异性。在一些实施方案中，在CDR结构域内进行不超过一至五个保守氨基酸替换。在另一些实施方案中，在CDR结构域内进行不超过一至三个保守氨基酸替换。在另一些实施方案中，CDR结构域是CDR H3和/或CDR L3。

[0402] 修饰还包括糖基化和非糖基化多肽，以及具有其他翻译后修饰的多肽，例如用不同糖的糖基化、乙酰化和磷酸化。抗体在其恒定区的保守位置被糖基化(Jefferis和Lund，1997，Chem.Immunol.65:111-128;Wright和 Morrison，1997，TibTECH 15:26-32)。免疫球蛋白的寡糖侧链影响蛋白质的功能(Boyd等，1996，Mol.Immunol.32:1311-1318;Wittwe和Howard，1990， Biochem.29:4175-4180)以及糖蛋白的部分之间的分子内相互作用，其可影响糖蛋白的构象和呈现的三维表面(Hefferis和Lund，supra;Wyss和 Wagner，1996，Current Opin.Biotech.7:409-416)。基于特定的识别结构，寡糖也可用于将给定的糖蛋白靶向某些分子。据报道，抗体的糖基化影响抗体依赖性细胞毒性(ADCC)。特别地，据报道具有四环素调节的β(1,4)-N- 乙酰葡糖胺基转移酶III(GnTIII)(催化形成二等分GlcNAc的糖基转移酶)表达的CHO细胞具有改善的ADCC活性(Umana等，1999，Mature Biotech.17:176-180)。

[0403] 抗体的糖基化通常是N-连接的或O-连接的。N-连接的是指碳水化合物部分与天冬酰胺残基的侧链的连接。三肽序列天冬酰胺-X-丝氨酸、天冬酰胺-X-苏氨酸和天冬酰胺-X-半胱氨酸(其中X是除脯氨酸以外的任何氨基酸)是用于将碳水化合物部分酶促连接至天冬酰胺侧链的识别序列。因此，多肽中这些三肽序列中任一种的存在产生潜在的糖基化位点。O- 连接的糖基化是指将糖N-乙酰半乳糖胺、半乳糖或木糖之一连接至羟基氨基酸(最常见的是丝氨酸或苏氨酸)，尽管也可使用5-羟基脯氨酸或5- 羟基赖氨酸。

[0404] 通过改变氨基酸序列以使其含有一个或更多个上述三肽序列(用于 N-连接的糖基化位点)而方便地实现向抗体添加糖基化位点。该改变也可通过向原始抗体的序列添加或替换一个或更多个丝氨酸或苏氨酸残基来进行(用于O-连接的糖基化位点)。

[0405] 也可改变抗体的糖基化模式而不改变潜在的核苷酸序列。糖基化主要取决于用于表达抗体的宿主细胞。由于用于表达作为潜在治疗剂的重组糖蛋白(例如，抗体)的细胞类型很少是天然细胞，所以可预期抗体的糖基化模式的变化(参见例如Hse等，1997，J.Biol.Chem.272:9062-9070)。

[0406] 除了选择宿主细胞外，在重组生产抗体期间影响糖基化的因素包括生长模式、培养基配方、培养密度、氧合作用、pH、纯化方案等。已经提出了多种方法来改变特定宿主生物体中获得的糖基化模式，包括引入或过表达参与寡糖生产的某些酶(美国专利号5,047,335;5,510,261和5,278,299)。糖基化或某些类型的糖基化可从糖蛋白中酶促去除，例如使用内切糖苷酶 H(Endo H)、N-糖苷酶F、内切糖苷酶F1、内切糖苷酶F2、内切糖苷酶 F3。此外，重组宿主细胞可被遗传改造以在加工某些类型的多糖中具有缺陷。这些和类似的技术是本领域中公知的。

[0407] 其他修饰方法包括使用本领域中已知的偶联技术，包括但不限于酶促手段、氧化取代和螯合。例如，修饰可用于附接用于免疫测定的标签。经修饰G1多肽可使用本领域已建立的程序来制备，并且可使用本领域中已知的标准测定进行筛选，其中一些在下文和实施例中描述。

[0408] 在本发明的一些实施方案中，抗体包含经修饰恒定区，例如免疫惰性或部分惰性

的恒定区，例如其不触发补体介导的裂解，不刺激抗体依赖性细胞介导的细胞毒性(ADCC)或不激活小胶质细胞；或者在下列任何一种或更多种中具有降低的活性(与未经修饰抗体相比)：触发补体介导的裂解，刺激抗体依赖性细胞介导的细胞毒性(ADCC)或激活小胶质细胞。可使用恒定区的不同修饰来实现效应功能的最佳水平和/或组合。参见例如Morgan等，Immunology 86:319-324(1995)；Lund等，J.Immunology 157:4963-9 157:4963-4969(1996)；Idusogie等，J.Immunology 164:4178-4184(2000)；Tao等，J.Immunology 143:2595-2601(1989)；以及 Jefferis等，Immunological Reviews 163:59-76(1998)。在一些实施方案中，恒定区如以下中所述进行修饰：Eur.J.Immunol.(1999)29:2613-2624；PCT 申请号PCT/GB99/01441；和/或英国专利申请号9809951.8。在另一些实施方案中，抗体包含人重链IgG2恒定区，其包含以下突变：A330P331至 S330S331(参照野生型IgG2序列进行氨基酸编号)。Eur.J.Immunol.(1999) 29:2613-2624。在另一些实施方案中，将恒定区非糖基化以用于N-连接的糖基化。在一些实施方案中，通过使作为恒定区中N-糖基化识别序列一部分的糖基化氨基酸残基或侧翼残基突变，将恒定区非糖基化以用于N- 连接的糖基化。例如，可将N-糖基化位点N297突变为A、Q、K或H。参见Tao等，J.Immunology 143:2595-2601(1989)；以及Jefferis等， Immunological Reviews 163:59-76(1998)。在一些实施方案中，将恒定区非糖基化以用于N-连接的糖基化。可酶促(例如通过酶PNGase移除碳水化合物)或通过在糖基化缺陷型宿主细胞中表达来将恒定区非糖基化以用于 N-连接的糖基化。

[0409] 其他抗体修饰包括已经如1999年11月18日公开的PCT公开No.WO 99/58572中所述修饰的抗体。除了针对靶分子的结合结构域之外，这些抗体包含具有与人免疫球蛋白重链的全部或部分恒定结构域基本同源的氨基酸序列的效应结构域(effector domain)。这些抗体能够结合靶分子而不触发显著的补体依赖性裂解或细胞介导的靶标破坏。在一些实施方案中，效应结构域能够特异性结合FcRn和/或Fc γ RIIb。这些通常基于来自于两个或更多个人免疫球蛋白重链C_H2结构域的嵌合结构域。以这种方式修饰的抗体特别适用于长期抗体治疗，以避免对常规抗体治疗的炎性和其他不良反应。

[0410] 在一些实施方案中，本发明包括亲和力成熟的实施方案。例如，亲和力成熟的抗体可通过本领域中已知的方法产生(Marks等，1992， Bio/Technology，10:779-783；Barbas等，1994，Proc Nat.Acad.Sci，USA 91:3809-3813；Schier等，1995，Gene，169:147-155；Yelton等，1995，J. Immunol.，155:1994-2004；Jackson等，1995，J.Immunol.，154(7):3310-9； Hawkins等，1992，J.Mol.Biol.，226:889-896；和WO2004/058184)。

[0411] 以下方法可用于调节抗体的亲和力并用于表征CDR。表征抗体的 CDR和/或改变(例如改善)多肽(例如抗体)的结合亲和力的一种方法被称为“文库扫描诱变”。通常来说，文库扫描诱变如下工作。使用本领域识别方法将CDR中的一个或更多个氨基酸位置替换成两个或更多个(例如，3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、或20个)氨基酸。这产生小的克隆文库(在一些实施方案中，所分析的每个氨基酸位置一个)，每个具有两个或更多个成员的复杂性(如果在每个位置替换两个或更多个氨基酸)。通常来说，文库还包括包含天然(未经替换)氨基酸的克隆。筛选来自每个文库的少量克隆(例如约20-80个克隆)(取决于文库的复杂性)与靶多肽(或其他结合靶标)的结合亲和力，并且鉴定具有提高的结合、相同的结合、降低的结合或无结合的候选物。确定结合亲和力的方法是本领域中公知的。可使用Biacore表面等离子体共振分析确定结合亲和力，其检测约2倍或更大的结合

亲和力差异。当起始抗体已经以相对高的亲和力(例如约10nM或更低的K_D)结合时，Biacore是特别可用的。使用Biacore表面等离子体共振的筛选描述于本文中的实施例中。

[0412] 可使用Kinexa Biocensor、闪烁邻近测定(scintillation proximity assay)、ELISA、ORIGEN免疫测定(IGEN)、荧光猝灭、荧光转移和/或酵母展示来确定结合亲和力。还可使用合适的生物测定来筛选结合亲和力。

[0413] 在一些实施方案中，使用本领域公认的诱变方法(其中一些在本文中描述)，用全部20种天然氨基酸替换CDR中的每个氨基酸位置(在一些实施方案中，一次一个)。这产生小的克隆文库(在一些实施方案中，所分析的每个氨基酸位置一个)，每个具有20个成员的复杂性(如果在每个位置替换所有20种氨基酸)。

[0414] 在一些实施方案中，待筛选的文库在两个或更多个位置包含替换，其可在相同的CDR中或在两个或更多个CDR中。因此，文库可包含一个 CDR中两个或更多个位置的替换。文库可包含两个或更多个CDR中的两个或更多个位置的取代。所述文库可包含在3、4、5或更多个位置的替换，所述位置存在于2、3、4、5或6个CDR中。可使用低冗余密码子来进行替换。参见例如Balint等，(1993)Gene 137(1):109-18)的表2。

[0415] CDR可以是CDRH3和/或CDRL3。CDR可以是CDRL1、CDRL2、 CDRL3、CDRH1、CDRH2和/或CDRH3中的一种或更多种。CDR可以是Kabat CDR、Chothia CDR或延伸的CDR。

[0416] 可对具有改善的结合的候选物进行测序，从而鉴定导致亲和力改善的 CDR替换突变体(也称为“改善的”替换)。也可对结合的候选物进行测序，从而鉴定保留结合的CDR替换。

[0417] 可进行多轮筛选。例如，具有改善的结合的候选物(各自包含在一个或更多个CDR的一个或更多个位置处的氨基酸替换)也可用于设计在每个改善的CDR位置(即，替换突变体表现出改善的结合的CDR中的氨基酸位置)处包含至少原始和替换的氨基酸的第二文库。下面将进一步讨论该文库的制备、以及筛选或选择。

[0418] 文库扫描诱变还提供了表征CDR的手段，其中具有改善的结合、相同的结合、降低的结合或不结合的克隆的频率也提供了关于每个氨基酸位置对于抗体-抗原复合物稳定性的重要性。例如，如果CDR的位置在改变为全部20种氨基酸时保持结合，则该位置被鉴定为抗原结合不太可能需要的位置。相反，如果CDR的位置在仅小百分比的替换中保持结合，则该位置被鉴定为对CDR功能重要的位置。因此，文库扫描诱变方法产生关于CDR中可改变为许多不同氨基酸(包括所有20种氨基酸)的位置以及CDR中不可改变或仅可改变为少数氨基酸的位置的信息。

[0419] 具有改善的亲和力的候选物可组合在第二文库中，该文库包括改善的氨基酸，该位置的原始氨基酸，并且在该位置可进一步包括额外的替换，这取决于期望的文库复杂性，或允许使用期望的筛选或选择方法。另外，如果期望，相邻的氨基酸位置可被随机化成至少两个或更多个氨基酸。相邻氨基酸的随机化可允许突变CDR中的额外的构象灵活性，这可进而允许或促进引入更大数目的改善突变。该文库还可在第一轮筛选中没有显示出改善的亲和力的位置处包含替换。

[0420] 使用本领域中已知的任何方法(包括使用Biacore表面等离子体共振分析进行筛选)以及使用本领域中已知的用于选择的任何方法进行选择 (包括噬菌体展示、酵母展示和核糖体展示)来筛选或选择第二文库的具有改善的和/或改变的结合亲和力的文库成员。

[0421] 在一些实施方案中，本发明还涵盖包含来自本发明的抗体(例如G1) 或多肽的一个或更多个片段或区域的融合蛋白。在一个实施方案中，提供了融合多肽，其包含SEQ ID NO:2中所示可变轻链区(图5)的至少10 个连续氨基酸和/或SEQ ID NO:1中所示可变重链区(图5)的至少10个氨基酸。在另一些实施方案中，提供了融合多肽，其包含SEQ ID NO:2中所示的可变轻链区(图5)的至少约10个、至少约15个、至少约20 个、至少约25个、或至少约30个连续氨基酸，和/或SEQ ID NO:1中所示的可变重链区(图5)的至少约10个、至少约15个、至少约20个、至少约25个、或至少约30个连续氨基酸。在另一个实施方案中，融合多肽包含如图5的SEQ ID NO:2和SEQ ID NO:1中所示G1的轻链可变区和 /或重链可变区。在另一个实施方案中，融合多肽包含G1的一个或更多个 CDR。在另一些实施方案中，融合多肽包含抗体G1的CDR H3和/或CDR L3。为了本发明的目的，G1融合蛋白包含一个或更多个G1抗体和在天然分子中其不连接的其他氨基酸序列，例如来自其他区域的异源序列或同源序列。示例性的异源序列包括但不限于“标签”，例如FLAG标签或6His 标签(SEQ ID NO:56)。标签是本领域中公知的。

[0422] G1融合多肽可通过本领域中已知的方法产生(例如合成或重组)。通常来说，通过使用本文中所述的重组方法制备和表达编码它们的多核苷酸来制备本发明的G1融合蛋白，尽管其也可通过本领域中已知的其他方式制备，包括例如化学合成。

[0423] 在一些方面，本发明还提供了包含与有助于与固体支持物偶联的试剂 (例如生物素或亲和素)缀合(例如连接)的来自于G1的抗体或多肽的组合物。为了简单起见，一般提及G1或抗体时理解为这些方法适用于本文中所述的任何CGRP结合实施方案。缀合通常是指连接如本文中所述的这些组分。连接(通常至少为了施用而将这些组分固定在接近的缔合中)可以以任何多种方式实现。例如，当药剂与抗体各自具有能够彼此反应的取代基时，它们之间的直接反应是可能的。例如，一个上的亲核基团(例如氨基或巯基)可以能够与另一个上的含羰基的基团(例如酸酐或酰基卤) 或者含良好离去基团(例如，卤化物)的烷基反应。

[0424] 抗体或多肽可与标记试剂(或者称为“标记”)(例如荧光分子、放射性分子或本领域中已知的任何其他标记)连接。本领域中已知通常提供(直接或间接)信号的标记。

[0425] 在一些实施方案中，本发明还提供了包含抗体G1和/或本文中所述的任何或全部抗体或多肽的组合物(包括药物组合物)和药盒。

[0426] 在一些实施方案中，本发明还提供了编码本发明的抗体和多肽(包括包含图5中所示轻链和重链可变区的多肽序列的抗体)的分离的多核苷酸，以及包含所述多核苷酸的载体和宿主细胞。

[0427] 在一些实施方案中，本发明提供了包含编码以下任意种的多核苷酸的多核苷酸(或组合物，包括药物组合物)：(a)抗体G1或其在表6中所示变体；(b)抗体G1或其在表6中所示变体的片段或区域；(c)抗体G1 或其在表6中所示变体的轻链；(d)抗体G1或其在表6中所示变体的重链；(e)来自抗体G1或其在表6中所示变体的轻链和/或重链的一个或更多个可变区；(f)抗体G1或其在表6中所示变体的一个或更多个CDR(一个、两个、三个、四个、五个或六个CDR)；(g)来自抗体G1的重链的CDR H3；(h)来自抗体G1或其在表6中所示变体的轻链的CDR L3；(i) 来自抗体G1或其在表6中所示变体的轻链的三个CDR；(j)来自抗体 G1或其在表6中所示变体的重链的三个CDR；(k)来自抗体G1或其在表6中所示变体的轻链的三个CDR和重链的三个CDR；以及(l)包含(b) 至(k)中任一个的抗体。在一些实施方案中，多核苷酸

包含SEQ ID NO:9 和SEQ ID NO:10中所示任一种或两种多核苷酸。

[0428] 在另一个方面,本发明提供了编码任何本文中所述抗体(包括抗体片段)和多肽(例如具有受损效应功能的抗体和多肽)中的任意种的多核苷酸。多核苷酸可通过本领域中已知的方法制备。

[0429] 在另一个方面,本发明提供了包含本发明的任何多核苷酸的组合物 (例如药物组合物)。在一些实施方案中,组合物包含含有编码如本文中所述的G1抗体的多核苷酸的表达载体。在另一些实施方案中,组合物包含含有编码本文中所述的任何抗体或多肽的多核苷酸的表达载体。在另一些实施方案中,组合物包含SEQ ID NO:9和SEQ ID NO:10中所示多核苷酸中的任一种或两种。本文中进一步描述了表达载体和多核苷酸组合物的施用。

[0430] 在另一个方面,本发明提供了制备本文中所述的任何多核苷酸的方法。

[0431] 与任何此类序列互补的多核苷酸也涵盖在本发明中。多核苷酸可以是单链(编码链或反义链)或双链,并且可以是DNA(基因组、cDNA或合成的)或RNA分子。RNA分子包括含有内含子并以一对一的方式对应于DNA分子的HnRNA分子和不含内含子的mRNA分子。额外的编码或非编码序列可以但不一定存在于本发明的多核苷酸内,并且多核苷酸可以但不一定与其他分子和/或支持材料连接。

[0432] 多核苷酸可包含天然序列(即,编码抗体或其部分的内源序列)或可包含这样的序列的变体。多核苷酸变体包含一个或更多个替换、添加、缺失和/或插入,使得相对于天然免疫反应性分子,编码的多肽的免疫反应性不减弱。通常可如本文中所述评估对编码的多肽的免疫反应性的影响。变体与编码天然抗体或其一部分的多核苷酸序列优选具有至少约70%的同一性、更优选至少约80%的同一性、并且最优选至少约90%的同一性。

[0433] 如下文所述,如果在用于最大对应性比对时两个序列中的核苷酸或氨基酸的序列相同,则认为两个多核苷酸或多肽序列是“同一的”。两个序列之间的比较通常通过对比较窗口上的序列进行比较来进行,以鉴定和比较序列相似性的局部区域。本文中使用的“比较窗口”是指至少约20个连续位置(通常30至约75个、40至约50个),其中序列可与具有相同数目的连续位置的参考序列在两个序列最佳比对之后进行比较。

[0434] 用于比较的序列的最佳比对可使用Lasergene生物信息学软件套 (DNASTAR,Inc.,Madison,WI)中的Megalign程序使用默认参数进行。该程序体现了以下参考文献中描述的数种比对方案:Dayhoff,M.O.(1978) A model of evolutionary change in proteins-Matrices for detecting distant relationships.In Dayhoff,M.O.(编) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation,Washington DC第5卷,增刊3,第345-358页;Hein J.,1990,Unified Approach to Alignment and Phylogenes 第626-645页Methods in Enzymology第183卷,Academic Press,Inc.,San Diego,CA;Higgins,D.G和Sharp,P.M.,1989,CABIOS 5:151-153;Myers,E.W.和Muller W.,1988,CABIOS 4:11-17;Robinson,E.D.,1971,Comb. Theor.11:105;Santou,N.,Nes,M.,1987,Mol.Biol.Evol.4:406-425;Sneath, P.H.A.和Sokal,R.R.,1973,Numerical Taxonomy the Principles and Practice of Numerical Taxonomy,Freeman Press,San Francisco,CA;Wilbur,W.J.和 Lipman,D.J.,1983,Proc.Natl.Acad.Sci.USA 80:726-730。

[0435] 优选地,通过比较至少20个位置的比较窗口上的两个最佳比对的序列来确定“序

列同一性的百分比”,其中与参考序列(其不包含添加或缺失) 相比,比较窗口中多核苷酸或多肽序列的一部分可包含20%或更少、通常 5%至15%、或10%至12%的添加或缺失(即,空位),以用于两个序列的最佳比对。如下计算百分比:确定两个序列中出现相同核酸碱基或氨基酸残基的位置数来得到匹配位置的数目,用匹配位置的数目除以参考序列中位置的总数(即,窗口尺寸)并将结果乘以100,以得到序列同一性的百分比。

[0436] 变体也可以或作为替选地与天然基因或其一部分或互补物基本上同源。这样的多核苷酸变体能够在中等严格条件下与编码天然抗体的天然 DNA序列(或互补序列)杂交。

[0437] 合适的“中等严格条件”包括在5×SSC,0.5%SDS,1.0mM EDTA(pH 8.0)溶液中预洗;在5×SSC中于50℃-65℃下杂交过夜;随后在65℃下洗涤两次,每次用含有0.1%SDS的2X、0.5X和0.2X SSC洗涤20分钟。

[0438] 本文中使用的“高度严格条件”或“高严格性条件”是以下那些:(1)采用低离子强度和高温进行洗涤,例如0.015M氯化钠/0.0015M柠檬酸钠 /0.1%十二烷基硫酸钠,50℃;(2)在杂交过程中使用变性剂,例如甲酰胺,例如50%(v/v)甲酰胺,其具有0.1%牛血清白蛋白/0.1%Ficoll/0.1%聚乙烯吡咯烷酮/50mM pH 6.5的磷酸钠缓冲液,具有750mM氯化钠、75 mM柠檬酸钠,42℃;或(3)使用50%甲酰胺,5×SSC(0.75M NaCl, 0.075M柠檬酸钠),50mM磷酸钠(pH 6.8),0.1%焦磷酸钠,5×Denhardt 溶液,超声处理的鲑鱼精子DNA(50μg/ml),0.1%SDS和10%葡聚糖硫酸酯,42℃,在42℃下在0.2×SSC(氯化钠/柠檬酸钠)中和55℃下50%甲酰胺中洗涤,然后高度严格性洗涤由在55℃下含有EDTA的0.1×SSC 组成。本领域技术人员将认识到如何根据需要调整温度、离子强度等以适应例如探针长度等的因素。

[0439] 本领域普通技术人员将理解,由于遗传密码的简并性,存在许多编码如本文中所述的多肽的核苷酸序列。这些多核苷酸中的一些与任何天然基因的核苷酸序列具有最小的同源性。尽管如此,本发明特别考虑了由于密码子使用的差异而变化的多核苷酸。此外,包含本文中提供的多核苷酸序列的基因的等位基因也在本发明的范围内。等位基因是由于核苷酸的一个或更多个突变(例如缺失、添加和/或替换)而改变的内源基因。得到的 mRNA和蛋白质可以但不一定具有改变的结构或功能。可使用标准技术 (例如杂交、扩增和/或数据库序列比较)来鉴定等位基因。

[0440] 本发明的多核苷酸可使用化学合成、重组方法或PCR获得。化学多核苷酸合成的方法是本领域中公知的并且不需要在本文中详细描述。本领域技术人员可使用本文中提供的序列和商业DNA合成仪来产生期望的 DNA序列。

[0441] 对于使用重组方法制备多核苷酸,可将包含期望序列的多核苷酸插入到合适的载体中,并且可将载体继而导入合适的宿主细胞中用于复制和扩增,如本文中进一步讨论的。可通过本领域中已知的任何方式将多核苷酸插入宿主细胞中。通过直接摄取、胞吞、转染、F-接合或电穿孔导入外源多核苷酸来转化细胞。一旦导入,外源多核苷酸可作为非整合载体(例如质粒)维持在细胞内或整合到宿主细胞基因组中。如此扩增的多核苷酸可通过本领域中公知的方法从宿主细胞中分离。参见例如Sambrook等 (1989)。

[0442] 或者,PCR允许复制DNA序列。PCR技术是本领域中公知的,并描述于美国专利No.4,683,195、4,800,159、4,754,065和4,683,202以及PCR:The Polymerase Chain Reaction,Mullis等编,Birkauswer Press,Boston(1994) 中。

[0443] 可通过将分离的DNA用在合适的载体中并将其插入合适的宿主细胞中来获得RNA。

当细胞复制并且DNA被转录成RNA时，可随后使用本领域技术人员公知的方法分离RNA，如Sambrook等，(1989)中所述。

[0444] 合适的克隆载体可根据标准技术构建，或者可从本领域中可用的大量克隆载体中选择。尽管选择的克隆载体可根据预期使用的宿主细胞而变化，但可用的克隆载体将通常具有自我复制的能力，可具有特定限制性内切核酸酶的单个靶标，和/或可携带用于标记的基因，所述标记可用于选择含有载体的克隆。合适的例子包括质粒和细菌病毒，例如pUC18、pUC19、Bluescript(例如pBS SK+)及其衍生物、mp18、mp19、pBR322、 pMB9、ColE1、pCR1、RP4、噬菌体DNA和穿梭载体(例如pSA3和pAT28)。这些和许多其他克隆载体可从商业供应商(例如BioRad、Strategene和 Invitrogen)获得。

[0445] 表达载体通常是可复制的多核苷酸构建体，其包含根据本发明各个方面中的任一个的多核苷酸。意味着表达载体在宿主细胞必须可作为附加体或作为染色体DNA的组成部分进行复制。合适的表达载体包括但不限于质粒、病毒载体(包括腺病毒、腺相关病毒、逆转录病毒)、黏粒以及PCT 公开No.WO 87/04462中公开的表达载体。载体组分可通常可包括但不限于以下中的一种或更多种：信号序列；复制起点；一个或更多个标记基因；合适的转录控制元件(例如启动子、增强子和终止子)。为了表达(即，翻译)，通常还需要一个或更多个翻译控制元件，例如核糖体结合位点、翻译起始位点和终止密码子。

[0446] 含有目的多核苷酸的载体可通过许多适当的手段中的任何一种导入宿主细胞，包括电穿孔，使用氯化钙、氯化铷、磷酸钙、DEAE-葡聚糖或其他物质的转染；微弹轰击(microprojectile bombardment)；脂质体转染；以及感染(例如，其中载体是感染剂，例如牛痘病毒)。导入载体或多核苷酸的选择将通常取决于宿主细胞的特征。

[0447] 在一些方面，本发明还提供了包含本文中所述的任何多核苷酸的宿主细胞。任何能够过表达异源DNA的宿主细胞可用于分离编码目的抗体、多肽或蛋白质的基因。哺乳动物宿主细胞的非限制性实例包括但不限于 COS、HeLa和CHO细胞。还参见PCT公开No.WO 87/04462。合适的非哺乳动物宿主细胞包括原核生物(例如大肠杆菌或枯草芽孢杆菌(B.subtillis))和酵母(例如酿酒酵母(S.cerevisae)、粟酒裂殖酵母(S.pombe)；或乳酸克鲁维酵母(K.lactis))。优选地，宿主细胞表达cDNA，其水平为宿主细胞中相应内源目的抗体或蛋白质(如果存在的话)的约5倍高，更优选10倍高，甚至更优选20倍高。筛选宿主细胞与Aθ1-40的特异性结合通过免疫测定法或FACS进行。可鉴定过表达目的抗体或蛋白质的细胞。

[0448] D.组合物

[0449] 在一些实施方案中，用于本发明方法的组合物包含有效量的本文中所述抗体(例如，抗CGRP拮抗剂抗体、调节CGRP途径的单克隆抗体)或来自于抗体的多肽。此类组合物的实例以及如何配制也在前面的部分和下文中进行描述。在一个实施方案中，组合物还包含CGRP拮抗剂。在一些实施方案中，组合物包含调节CGRP途径的一种或更多种单克隆抗体。在一些实施方案中，组合物包含一种或更多种抗CGRP拮抗剂抗体。在一些实施方案中，抗CGRP拮抗剂抗体识别人CGRP。在一些实施方案中，抗 CGRP拮抗剂抗体是人源化的。在一些实施方案中，抗CGRP拮抗剂抗体包含不引发不想要的或不期望的免疫应答(例如抗体介导的裂解或 ADCC)的恒定区。在一些实施方案中，抗CGRP拮抗剂抗体包含抗体 G1的一个或更多个CDR(例如来自G1的一个、两个、三个、四个、五个或在一些实施方案中全部六个CDR)。在一些实施方案中，抗CGRP拮抗剂抗体是人的。

[0450] 应理解,组合物可包含多于一种抗体(例如,多于一种抗CGRP拮抗剂抗体——识别CGRP的不同表位的抗CGRP拮抗剂抗体的混合物)。另一些示例性组合物包含多于一种识别相同表位的抗CGRP拮抗剂抗体,或者与CGRP的不同表位结合的不同种类的抗CGRP拮抗剂抗体。

[0451] 组合物还可包含可药用载体、赋形剂或稳定剂(Remington:The Science and practice of Pharmacy第20版.(2000)Lippincott Williams和 Wilkins,Ed.K.E.Hoover)。可接受的载体、赋形剂或稳定剂在使用的剂量和浓度下对接受者无毒。抗体的治疗制剂可包含一种或更多种可药用载体、赋形剂或稳定剂,此类物质的非限制性实例包括缓冲剂,例如磷酸盐、柠檬酸盐和其他有机酸;盐,例如氯化钠;抗氧化剂,包括抗坏血酸和甲硫氨酸;防腐剂(例如十八烷基二甲基苄基氯化铵;六甲铵氯化物;苯扎氯铵,苄索氯铵;酚、丁基醇或苯甲醇;对羟基苯甲酸烷基酯,如对羟基苯甲酸甲酯或对羟基苯甲酸丙酯;儿茶酚;间苯二酚;环己醇;3-戊醇;以及间甲酚);低分子量(少于约10个残基)多肽;蛋白质,例如血清白蛋白、明胶或免疫球蛋白;亲水聚合物,例如聚乙烯吡咯烷酮;氨基酸(例如,浓度为0.1mM至100mM、0.1mM至1mM、0.01mM至50mM、1 mM至50mM、1mM至30mM、1mM至20mM、10mM至25mM),例如甘氨酸、谷氨酰胺、甲硫氨酸、天冬酰胺、组氨酸、精氨酸或赖氨酸;单糖、二糖和其他碳水化合物,包括葡萄糖、甘露糖或糊精;螯合剂(例如,浓度为0.001mg/mL至1mg/mL、0.001mg/mL至1mg/mL、0.001 mg/mL至0.1mg/mL、0.001mg/mL至0.01mg/mL),如EDTA(例如,二水合EDTA二钠);糖(例如,浓度为1mg/mL至500mg/mL、10mg/mL 至200mg/mL、10mg/mL至100mg/mL、50mg/mL至150mg/mL),例如蔗糖、甘露糖醇、海藻糖或山梨糖醇;成盐反离子,例如钠;金属配合物 (例如,Zn-蛋白质配合物);和/或非离子表面活性剂(例如,浓度为0.01 mg/mL至10mg/mL、0.01mg/mL至1mg/mL、0.1mg/mL至1mg/mL、 0.01mg/mL至0.5mg/mL),例如TWEEN™(例如,聚山梨酸酯(例如,聚山梨酸酯20、聚山梨酸酯40、聚山梨酸酯60、聚山梨酸酯80))、PLURONICS™或聚乙二醇(PEG)。本文中进一步描述了可药用赋形剂。

[0452] 抗体(例如,抗CGRP拮抗剂抗体)及其组合物也可与用于增强和/ 或补充药剂效力的其他试剂联合使用。

[0453] E.药盒

[0454] 在一个面,本发明还提供用于本发明方法的药盒。药盒可包含含有本文中所述抗体(例如,抗CGRP拮抗剂抗体(例如人源化抗体))或本文中所述多肽的一个或更多个容器,以及根据本文中所述任何方法使用的说明书。通常来说,这些说明书包含根据本文中所述的任何方法施用抗体以治疗、改善或预防创伤后头痛的描述。药盒可还包含基于鉴别个体是否具有创伤后头痛或个体是否处于患创伤后头痛之风险中来选择适合于治疗的个体的描述。在另一些实施方案中,说明书包含向处于患有创伤后头痛之风险中的个体施用抗体(例如,抗CGRP拮抗剂抗体)的描述。

[0455] 在一些实施方案中,抗体是人源化抗体。在一些实施方案中,抗体是人的。在另一些实施方案中,抗体是单克隆抗体。在一些实施方案中,抗体包含抗体G1的一个或更多个CDR(例如来自G1的一个、两个、三个、四个、五个或在一些实施方案中全部六个CDR)。

[0456] 涉及使用抗体(例如,抗CGRP拮抗剂抗体)的说明书一般包含关于用于预期治疗的剂量、给药方案和施用途径的信息。容器可以是单位剂量、散装包装(例如,多剂量包装)或亚单位剂量。药盒中提供的说明书通常是标签或包装插页(例如,药盒中包含的纸页)上的

书面说明书,但机器可读说明(例如,磁性或光学存储盘上携带的说明书)也是可接受的。

[0457] 标签或包装插页表明组合物用于治疗、改善和/或预防创伤后头痛。可提供说明书以实践本文中所述的任何方法。

[0458] 本发明的药盒在合适的包装中。合适的包装包括但不限于小瓶、瓶、罐、软包装(flexible packaging)(例如,密封的Mylar或塑料袋)等。还考虑与特定装置(例如吸入器、鼻腔施用装置(例如,雾化器))或输注装置(例如微型泵)组合使用的包装。药盒可具有无菌进入口(例如,容器可以是静脉内溶液袋或具有可被皮下注射针头刺穿的塞子的小瓶)。容器还可具有无菌进入口(例如,容器可以是静脉内溶液袋或具有可被皮下注射针头刺穿的塞子的小瓶)。组合物中的至少一种活性剂是抗CGRP拮抗剂抗体和/或调节CGRP途径的单克隆抗体。容器还可包含第二药物活性剂。

[0459] 药盒可任选地提供另外的组件,例如缓冲剂和解释性信息。通常来说,药盒包含容器和在容器上或与容器缔合的标签或包装插页。

[0460] 提供以下实施例来举例说明但不限制本发明。应理解,本文中描述的实施例和实施方案仅用于举例说明的目的,并且将向本领域技术人员表明对它们的多种修改和改变并且其包含在本申请的精神和范围内。本文引用的所有出版物、专利和专利申请在此出于所有目的通过引用整体并入本文,其程度如同每个单独的出版物、专利或专利申请被具体且单独地指示为如此通过引用并入。

[0461] 本发明的另一些方面和实施方案在以下编号的段落中列出:

[0462] 1.在对象中预防、治疗创伤后头痛或降低其发病率的方法,其包括向所述对象施用单克隆抗CGRP拮抗剂抗体。

[0463] 2.在对象中预防、治疗与创伤后头痛相关的至少一种继发症状或降低其发病率的方法,其包括向所述对象施用单克隆抗CGRP拮抗剂抗体。

[0464] 3.段落1或2所述的方法,其中所述单克隆抗体在创伤后头痛之前、期间和/或之后施用。

[0465] 4.段落3所述的方法,其中所述单克隆抗体在创伤后头痛发作之前(例如,在头部和/或颈部的创伤或损伤之后)施用。

[0466] 5.段落1或2所述的方法,其中所述单克隆抗体在头部和/或颈部的创伤或损伤之后立即施用。

[0467] 6.段落1或2所述的方法,其中向所述对象施用的所述单克隆抗体的量为约225mg至约1000mg。

[0468] 7.段落1或2所述的方法,其中所述单克隆抗体皮下施用。

[0469] 8.段落1或2所述的方法,其中所述单克隆抗体静脉内施用。

[0470] 9.段落1或2所述的方法,其中所述单克隆抗体以初始剂量(例如,负荷剂量)和一个或更多个额外剂量(例如,维持剂量)向所述对象施用。

[0471] 10.段落9所述的方法,其中所述初始剂量(例如,负荷剂量)和一个或更多个额外剂量(例如,维持剂量)的量相同或不同。

[0472] 11.段落1或2所述的方法,其中所述初始剂量(例如,负荷剂量) 和一个或更多个额外剂量(例如,维持剂量)以相同的方式例如皮下或静脉内施用。

[0473] 12.段落1或2所述的方法,其中所述一个或更多个额外剂量以与所述初始剂量(例

如,负荷剂量)不同的方式施用,例如所述初始剂量静脉内施用并且所述一个或更多个额外剂量(例如,维持剂量)皮下施用。

[0474] 13.段落1或2所述的方法,其中所述单克隆抗体每月、每季度或单次剂量施用。

[0475] 14.段落1或2所述的方法,其中所述单克隆抗体每月施用。

[0476] 15.段落1或2所述的方法,其中所述单克隆抗体每月施用持续三个月或更多个月。

[0477] 16.段落1或2所述的方法,其中给药方案包括皮下施用约675mg 的初始抗体剂量,随后皮下施用约225mg的每月抗体剂量持续约2个月,例如约3个月、4个月、5个月、6个月或12个月。

[0478] 17.段落1或2所述的方法,其中给药方案包括经过以约60分钟的输注静脉内施用约900mg的初始剂量,随后每季度以约60分钟的输注静脉内施用约900mg的剂量持续约1年,例如2年、3年、4年或5年。

[0479] 18.段落1或2所述的方法,其中所述治疗或降低可包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用,降低6项头痛影响测试(HIT-6)失能评分、改善12项简式健康调查(SF-12)评分(Ware等,Med Care 4:220-233,1996)、降低患者变化总体印象(PGIC)评分(Hurst等,J Manipulative Physiol Ther 27:26-35,2004)、改善运动震荡评估工具3(SCAT-3)评分(McCrory等 British Journal of Spots Medicine 47:263-266,2013)、或其任意组合。

[0480] 19.段落18所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛小时降低40个或更多个小时(例如,45、50、55、60、65、70、75、80或更多)。

[0481] 20.段落18所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛小时降低25%或更多(例如,30%、 35%、40%、45%、50%或更多)。

[0482] 21.段落18所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛日降低三天或更多天(例如,3、4、 5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20天或更多天)。

[0483] 22.段落1所述的方法,其中所述施用可包括利用包含一定剂量所述单克隆抗体的预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器。

[0484] 23.段落1所述的方法,其中所述单克隆抗体以至少150mg/mL的浓度配制。

[0485] 24.段落1所述的方法,其中所述单克隆抗体以小于2mL、例如约 1.5mL的体积施用。

[0486] 25.段落1所述的方法,其中所述方法还包括与所述单克隆抗体同时或先后向所述对象施用第二药剂。

[0487] 26.段落1所述的方法,其中所述第二药剂是5-HT1激动剂、曲普坦类、麦角生物碱类和非甾体抗炎药中的任一种。在一些实施方案中,所述第二药剂是对象预防性摄取的药剂。

[0488] 27.段落1所述的方法,其中在施用所述单克隆抗体之后,所述对象对所述第二药剂的每月使用降低至少15%。

[0489] 28.段落1所述的方法,其中所述第二药剂是曲普坦类。

[0490] 29.段落1所述的方法,其中所述对象是人。

[0491] 30.段落1所述的方法,其中所述单克隆抗体是人单克隆抗体或人源化单克隆抗体。

[0492] 31.段落1所述的方法,其中所述单克隆抗体是人源化单克隆抗体。

[0493] 32.段落1所述的方法,其中所述单克隆抗体包含例如IgG1、IgG2、IgG3和IgG4的抗体的重链恒定区。

[0494] 33.段落1所述的方法,其中所述单克隆抗体包含:(a)具有如SEQ ID NO:3所示CDR H1、如SEQ ID NO:4所示CDR H2、如SEQ ID NO:5 所示CDR H3、如SEQ ID NO:6所示CDR L1、如SEQ ID NO:7所示CDR L2和如SEQ ID NO:8所示CDR L3的抗体;或(b)如表6中所示的根据(a)的抗体的变体。

[0495] 34.段落1或2所述的方法,其中所述创伤后头痛是急性的或持久性的。

[0496] 35.段落1或2所述的方法,其中所述创伤后头痛是持久性的。

[0497] 36.用于根据前述段落中任一段落的组合物。

[0498] 1A.在对象中预防、治疗创伤后头痛或降低其发病率的方法,其包括向所述对象施用治疗有效量的单克隆抗CGRP拮抗剂抗体,其中所述单克隆抗体以约675mg至约900mg的初始负荷剂量静脉内或皮下施用,随后以一个月的间隔皮下施用约225mg的后续维持剂量。

[0499] 2A.在对象中预防、治疗与创伤后头痛相关的至少一种继发症状或降低其发病率的方法,其包括向所述对象施用治疗有效量的单克隆抗CGRP 拮抗剂抗体,其中所述单克隆抗体以约675mg至约900mg的初始负荷剂量静脉内施用,随后以一个月的间隔皮下施用约225mg的后续维持剂量。

[0500] 3A.段落1A或2A所述的方法,其中所述单克隆抗体在创伤后头痛之前、期间和/或之后施用。

[0501] 4A.段落3A所述的方法,其中所述单克隆抗体在创伤后头痛发作之前(例如,在头部和/或颈部的创伤或损伤之后)施用。

[0502] 5A.段落1A或2A所述的方法,其中所述单克隆抗体在头部和/或颈部的创伤或损伤之后立即施用。

[0503] 6A.段落1A或2A所述的方法,其中给药方案包括皮下施用约675mg 的初始抗体剂量,随后皮下施用约225mg的每月抗体剂量持续约2个月,例如约3个月、4个月、5个月、6个月或12个月。

[0504] 7A.段落1A或2A所述的方法,其中所述治疗或降低可包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、降低6项头痛影响测试(HIT-6)失能评分、改善 12项简式健康调查(SF-12)评分(Ware等,Med Care 4:220-233,1996)、降低患者变化总体印象(PGIC)评分(Hurst等,J Manipulative Physiol Ther 27:26-35,2004)、改善运动震荡评估工具3(SCAT-3)评分(McCrory等, British Journal of Spots Medicine 47:263-266,2013)、或其任意组合。

[0505] 8A.段落1A或2A所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛小时降低40个或更多个小时(例如,45、50、55、60、65、70、75、80或更多)。

[0506] 9A.段落1A或2A所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛小时降低25%或更多(例如, 30%、35%、40%、45%、50%或更

多)。

[0507] 10A.段落1A或2A所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛日降低三天或更多天(例如, 3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20 天或更多天)。

[0508] 11A.段落1A所述的方法,其中所述施用可包括利用包含一定剂量所述单克隆抗体的预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器。

[0509] 12A.段落1A所述的方法,其中所述单克隆抗体以至少150mg/mL 的浓度配制。

[0510] 13A.段落1A所述的方法,其中所述单克隆抗体以小于2mL、例如约1.5mL的体积施用。

[0511] 14A.段落1A所述的方法,其中所述方法还包括与所述单克隆抗体同时或先后向所述对象施用第二药剂。

[0512] 15A.段落1A所述的方法,其中所述第二药剂是5-HT1激动剂、曲普坦类、麦角生物碱类和非甾体抗炎药中的任一种。在一些实施方案中,第二药剂是对象预防性摄取的药剂。

[0513] 16A.段落1A所述的方法,其中在施用所述单克隆抗体之后,所述对象对所述第二药剂的每月使用降低至少15%。

[0514] 17A.段落1A所述的方法,其中所述第二药剂是曲普坦类。

[0515] 18A.段落1A所述的方法,其中所述对象是人。

[0516] 19A.段落1A所述的方法,其中所述单克隆抗体是人单克隆抗体或人源化单克隆抗体。

[0517] 20A.段落1A所述的方法,其中所述单克隆抗体是人源化单克隆抗体。

[0518] 21A.段落1A所述的方法,其中所述单克隆抗体包含例如IgG1、IgG2、 IgG3和IgG4的抗体的重链恒定区。

[0519] 22A.段落1A所述的方法,其中所述单克隆抗体包含:(a)具有如 SEQ ID NO:3所示CDR H1、如SEQ ID NO:4所示CDR H2、如SEQ ID NO:5所示CDR H3、如SEQ ID NO:6所示CDR L1、如SEQ ID NO:7所示CDR L2、和如SEQ ID NO:8所示CDR L3的抗体;或(b)如表6中所示的根据(a)的抗体的变体。

[0520] 23A.段落1A或2A所述的方法,其中所述创伤后头痛是急性的或持久性的。

[0521] 24A.段落1A或2A所述的方法,其中所述创伤后头痛是持久性的。

[0522] 25A.用于根据前述段落中任一段落的组合物。

[0523] 1B.在对象中预防、治疗创伤后头痛或降低其发病率的方法,其包括向所述对象施用治疗有效量的单克隆抗CGRP拮抗剂抗体,其中所述单克隆抗体以约675mg至约900mg的剂量每季度一次静脉内施用。

[0524] 2B.在对象中预防、治疗与创伤后头痛相关的至少一种继发症状或降低其发病率的方法,其包括向所述对象施用治疗有效量的单克隆抗CGRP 拮抗剂抗体,其中所述单克隆抗体以约675mg至约900mg的剂量每季度一次静脉内施用。

[0525] 3B.段落1B或2B所述的方法,其中所述单克隆抗体在创伤后头痛之前、期间和/或之后施用。

[0526] 4B.段落3B所述的方法,其中所述单克隆抗体在创伤后头痛发作之前(例如,在头部和/或颈部的创伤或损伤之后)施用。

[0527] 5B.段落1B或2B所述的方法，其中所述单克隆抗体在头部和/或颈部的创伤或损伤之后立即施用。

[0528] 6B.段落1B或2B所述的方法，其中给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量，随后每季度以约60分钟的输注静脉内施用约900mg的剂量持续约1年，例如2年、3年、4年或5年。

[0529] 7B.段落1B或2B所述的方法，其中所述治疗或降低可包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、降低6项头痛影响测试(HIT-6)失能评分、改善12项简式健康调查(SF-12)评分(Ware等，Med Care 4:220-233，1996)、降低患者变化总体印象(PGIC)评分(Hurst等，J Manipulative Physiol Ther 27:26-35，2004)、改善运动震荡评估工具3(SCAT-3)评分(McCrory等， British Journal of Spots Medicine 47:263-266，2013)、或其任意组合。

[0530] 8B.段落1B或2B所述的方法，其中相对于所述对象中的施用前水平，在所述施用之后所述对象经历的每月头痛小时降低40个或更多个小时(例如，45、50、55、60、65、70、75、80或更多)。

[0531] 9B.段落1B或2B所述的方法，其中相对于所述对象中的施用前水平，在所述施用之后所述对象经历的每月头痛小降低25％或更多(例如， 30％、35％、40％、45％、50％或更多)。

[0532] 10B.段落1B或2B所述的方法，其中相对于所述对象中的施用前水平，在所述施用之后所述对象经历的每月头痛日降低三天或更多天(例如 3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20 天或更多天)。

[0533] 11B.段落1B所述的方法，其中所述施用可包括利用包含一定剂量所述单克隆抗体的预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器。

[0534] 12B.段落1B所述的方法，其中所述单克隆抗体以至少150mg/mL 的浓度配制。

[0535] 13B.段落1B所述的方法，其中所述单克隆抗体以小于2mL、例如约1.5mL的体积施用。

[0536] 14B.段落1B所述的方法，其中所述方法还包括与所述单克隆抗体同时或先后向所述对象施用第二药剂。

[0537] 15B.段落1B所述的方法，其中所述第二药剂是5-HT1激动剂、曲普坦类、麦角生物碱类和非甾体抗炎药中的任一种。在一些实施方案中，所述第二药剂是对象预防性摄取的药剂。

[0538] 16B.段落1B所述的方法，其中在施用所述单克隆抗体之后，所述对象对所述第二药剂的每月使用降低至少15％。

[0539] 17B.段落1B所述的方法，其中所述第二药剂是曲普坦类。

[0540] 18B.段落1B所述的方法，其中所述对象是人。

[0541] 19B.段落1B所述的方法，其中所述单克隆抗体是人单克隆抗体或人源化单克隆抗体。

[0542] 20B.段落1B所述的方法，其中所述单克隆抗体是人源化单克隆抗体。

[0543] 21B.段落1B所述的方法，其中所述单克隆抗体包含例如IgG1、IgG2、 IgG3和IgG4的抗体的重链恒定区。

[0544] 22B.段落1B所述的方法,其中所述单克隆抗体包含:(a)具有如 SEQ ID NO:3所示CDR H1、如SEQ ID NO:4所示CDR H2、如SEQ ID NO:5所示CDR H3、如SEQ ID NO:6所示CDR L1、如SEQ ID NO:7所示CDR L2、和如SEQ ID NO:8所示CDR L3的抗体;或(b)如表6中所示的根据(a)的抗体的变体。

[0545] 23B.段落1B或2B所述的方法,其中所述创伤后头痛是急性的或持久性的。

[0546] 24B.段落1B或2B所述的方法,其中所述创伤后头痛是持久性的。

[0547] 25B.用于根据前述段落中任一段落的组合物。

[0548] 1C.在对象中预防、治疗创伤后头痛或降低其发病率的方法,其包括向所述对象施用治疗有效量的单克隆抗CGRP拮抗剂抗体,其中所述单克隆抗体以约675mg至约900mg的剂量每三个月间隔一次静脉内施用。

[0549] 2C.在对象中预防、治疗与创伤后头痛相关的至少一种继发症状或降低其发病率的方法,其包括向所述对象施用治疗有效量的单克隆抗CGRP 拮抗剂抗体,其中所述单克隆抗体以约675mg至约900mg的剂量每三个月间隔一次静脉内施用。

[0550] 3C.段落1C或2C所述的方法,其中所述单克隆抗体在创伤后头痛之前、期间和/或之后施用。

[0551] 4C.段落3C所述的方法,其中所述单克隆抗体在创伤后头痛发作之前(例如,在头部和/或颈部的创伤或损伤之后)施用。

[0552] 5C.段落1C或2C所述的方法,其中所述单克隆抗体在头部和/或颈部的创伤或损伤之后立即施用。

[0553] 6C.段落1C或2C所述的方法,其中给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量,随后每三个月以约60分钟的输注静脉内施用约900mg的剂量持续约1年,例如2年、3年、4年或5年。

[0554] 7C.段落1C或2C所述的方法,其中所述治疗或降低可包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、降低6项头痛影响测试(HIT-6)失能评分、改善 12项简式健康调查(SF-12)评分(Ware等,Med Care 4:220-233,1996)、降低患者变化总体印象(PGIC)评分(Hurst等,J Manipulative Physiol Ther 27:26-35,2004)、改善运动震荡评估工具3(SCAT-3)评分(McCrory等, British Journal of Spots Medicine 47:263-266,2013)、或其任意组合。

[0555] 8C.段落1C或2C所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛小时降低40个或更多个小时(例如,45、50、55、60、65、70、75、80或更多)。

[0556] 9C.段落1C或2C所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛小时降低25%或更多(例如,30%、35%、40%、45%、50%或更多)。

[0557] 10C.段落1C或2C所述的方法,其中相对于所述对象中的施用前水平,在所述施用之后所述对象经历的每月头痛日降低三天或更多天(例如, 3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20 天或更多天)。

[0558] 11C.段落1C所述的方法,其中所述施用可包括利用包含一定剂量所述单克隆抗体的预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器。

[0559] 12C.段落1C所述的方法，其中所述单克隆抗体以至少150mg/mL 的浓度配制。

[0560] 13C.段落1C所述的方法，其中所述单克隆抗体以小于2mL、例如约1.5mL的体积施用。

[0561] 14C.段落1C所述的方法，其中所述方法还包括与所述单克隆抗体同时或先后向所述对象施用第二药剂。

[0562] 15C.段落1C所述的方法，其中所述第二药剂是5-HT1激动剂、曲普坦类、麦角生物碱类和非甾体抗炎药中的任一种。在一些实施方案中，所述第二药剂是对象预防性摄取的药剂。

[0563] 16C.段落1C所述的方法，其中在施用所述单克隆抗体之后，所述对象对所述第二药剂的每月使用降低至少15％。

[0564] 17C.段落1C所述的方法，其中所述第二药剂是曲普坦类。

[0565] 18C.段落1C所述的方法，其中所述对象是人。

[0566] 19C.段落1C所述的方法，其中所述单克隆抗体是人单克隆抗体或人源化单克隆抗体。

[0567] 20C.段落1C所述的方法，其中所述单克隆抗体是人源化单克隆抗体。

[0568] 21C.段落1C所述的方法，其中所述单克隆抗体包含例如IgG1、IgG2、IgG3和IgG4的抗体的重链恒定区。

[0569] 22C.段落1C所述的方法，其中所述单克隆抗体包含：(a)具有如 SEQ ID NO:3所示CDR H1、如SEQ ID NO:4所示CDR H2、如SEQ ID NO:5所示CDR H3、如SEQ ID NO:6所示CDR L1、如SEQ ID NO:7所示CDR L2、和如SEQ ID NO:8所示CDR L3的抗体；或(b)如表6中所示的根据(a)的抗体的变体。

[0570] 23C.段落1C或2C所述的方法，其中所述创伤后头痛是急性的或持久性的。

[0571] 24C.段落1C或2C所述的方法，其中所述创伤后头痛是持久性的。

[0572] 25C.用于根据前述段落中任一段落的组合物。

[0573] 1D.在对象中预防、治疗创伤后头痛或降低其发病率的方法，其包括向所述对象施用治疗有效量的单克隆抗CGRP拮抗剂抗体，其中所述单克隆抗体以约675mg至约900mg的剂量每六个月间隔一次静脉内施用。

[0574] 2D.在对象中预防、治疗与创伤后头痛相关的至少一种继发症状或降低其发病率的方法，其包括向所述对象施用治疗有效量的单克隆抗CGRP 拮抗剂抗体，其中所述单克隆抗体以约675mg至约900mg的剂量每六个月间隔一次静脉内施用。

[0575] 3D.段落1D或2D所述的方法，其中所述单克隆抗体在创伤后头痛之前、期间和/或之后施用。

[0576] 4D.段落3D所述的方法，其中所述单克隆抗体在创伤后头痛发作之前(例如，在头部和/或颈部的创伤或损伤之后)施用。

[0577] 5D.段落1D或2D所述的方法，其中所述单克隆抗体在头部和/或颈部的创伤或损伤之后立即施用。

[0578] 6D.段落1D或2D所述的方法，其中给药方案包括以约60分钟的输注静脉内施用约900mg的初始剂量，随后每六个月以约60分钟的输注静脉内施用约900mg的剂量持续约1年，例如2年、3年、4年或5年。

[0579] 7D.段落1D或2D所述的方法，其中所述治疗或降低可包括降低任何严重程度的头痛小时数、降低任何严重程度的每月头痛日数、降低任何急性头痛药物的使用、降低6项头痛影响测试(HIT-6)失能评分、改善 12项简式健康调查(SF-12)评分(Ware等，Med Care 4:220-233，1996)、降低患者变化总体印象(PGIC)评分(Hurst等，J Manipulative Physiol Ther 27:26-35，2004)、改善运动震荡评估工具3(SCAT-3)评分(McCrory等， British Journal of Spots Medicine 47:263-266，2013)、或其任意组合。

[0580] 8D.段落1D或2D所述的方法，其中相对于所述对象中的施用前水平，在所述施用之后所述对象经历的每月头痛小时降低40个或更多个小时(例如，45、50、55、60、65、70、75、80或更多)。

[0581] 9D.段落1D或2D所述的方法，其中相对于所述对象中的施用前水平，在所述施用之后所述对象经历的每月头痛小时降低25％或更多(例如， 30％、35％、40％、45％、50％或更多)。

[0582] 10D.段落1D或2D所述的方法，其中相对于所述对象中的施用前水平，在所述施用之后所述对象经历的每月头痛日降低三天或更多天(例如， 3、4、5、6、7、8、9、10、11、12、13、14、15、16、17、18、19、20 天或更多天)。

[0583] 11D.段落1D所述的方法，其中所述施用可包括利用包含一定剂量所述单克隆抗体的预填充注射器、具有针安全装置之预填充注射器、注射笔或自动注射器。

[0584] 12D.段落1D所述的方法，其中所述单克隆抗体以至少150mg/mL 的浓度配制。

[0585] 13D.段落1D所述的方法，其中所述单克隆抗体以小于2mL、例如约1.5mL的体积施用。

[0586] 14D.段落1D所述的方法，其中所述方法还包括与所述单克隆抗体同时或先后向所述对象施用第二药剂。

[0587] 15D.段落1D所述的方法，其中所述第二药剂是5-HT1激动剂、曲普坦类、麦角生物碱类和非甾体抗炎药中的任一种。在一些实施方案中，所述第二药剂是对象预防性摄取的药剂。

[0588] 16D.段落1D所述的方法，其中在施用所述单克隆抗体之后，所述对象对所述第二药剂的每月使用降低至少15％。

[0589] 17D.段落1D所述的方法，其中所述第二药剂是曲普坦类。

[0590] 18D.段落1D所述的方法，其中所述对象是人。

[0591] 19D.段落1D所述的方法，其中所述单克隆抗体是人单克隆抗体或人源化单克隆抗体。

[0592] 20D.段落1D所述的方法，其中所述单克隆抗体是人源化单克隆抗体。

[0593] 21D.段落1D所述的方法，其中所述单克隆抗体包含例如IgG1、IgG2、 IgG3和IgG4的抗体的重链恒定区。

[0594] 22D.段落1D所述的方法，其中所述单克隆抗体包含：(a)具有如SEQ ID NO:3所示CDR H1、如SEQ ID NO:4所示CDR H2、如SEQ ID NO:5所示CDR H3、如SEQ ID NO:6所示CDR L1、如SEQ ID NO:7所示CDR L2、和如SEQ ID NO:8所示CDR L3的抗体；或(b)如表6中所示的根据(a)的抗体的变体。

[0595] 23D.段落1D或2D所述的方法，其中所述创伤后头痛是急性的或持久性的。

[0596] 24D.段落1D或2D所述的方法,其中所述创伤后头痛是持久性的。

[0597] 25D.用于根据前述段落中任一段落的组合物。

实施例

[0598] 实施例1:针对CGRP的单克隆抗体的产生和表征

[0599] 抗CGRP抗体的产生。为了产生对大鼠和人CGRP具有跨物种反应性的抗CGRP抗体,用佐剂中的25-100μg与KLH缀合的人α-CGRP或β-CGRP以多种间隔对小鼠进行免疫接种(每个足垫50μl,每只小鼠100 μl)。通常按照以下中的描述进行免疫接种:Geerligs HJ等,1989,J. Immunol.Methods 124:95-102;Kenney JS等,1989,J.Immunol.Methods 121:157-166;和Wicher K等,1989,Int.Arch.Allergy Appl.Immunol. 89:128-135。首先用CFA(完全弗氏佐剂)中的50μg与KLH缀合的人α-CGRP或β-CGRP对小鼠进行免疫接种。21天后,用IFA(不完全弗氏佐剂)中的25μg与KLH缀合的人β-CGRP(用于首次用人α-CGRP免疫接种的小鼠)或α-CGRP(用于首次用人β-CGRP免疫接种的小鼠)对小鼠进行第二次免疫接种。在第二次免疫接种之后23天后,用IFA中的25 μg与KLH缀合的大鼠α-CGRP对小鼠进行第三次免疫接种。十天之后,使用ELISA测试抗体滴度。在第三次免疫接种之后34天,用IFA中的25 μg肽(大鼠α-CGRP-KLH)进行第四次免疫接种。在第四次免疫接种之后32天,用100μg可溶性肽(大鼠α-CGRP)进行最后的加强。

[0600] 从经免疫接种小鼠获得脾细胞,并利用聚乙二醇1500与NSO骨髓瘤细胞以10:1的比例融合。将杂交物在含有20%马血清和2-草酰乙酸/丙酮酸/胰岛素(Sigma)的DMEM中平板接种到96孔板中,并开始次黄嘌呤 /氨基蝶呤/胸苷选择。在第8天,向所有孔中添加100μl含有20%马血清的DMEM。使用抗体捕获免疫测定来筛选杂交物的上清液。用类别特异性二抗进行抗体类别的确定。

[0601] 基于其与人和大鼠CGRP的结合来选择一组产生单克隆抗体的细胞系用于进一步表征。这些抗体和特征在下表2和3中示出。

[0602] 纯化和Fab片段制备。使用蛋白A亲和色谱从杂交瘤培养物的上清液纯化选择用于进一步表征的单克隆抗体。将上清液平衡至pH 8。然后将上清液加载至用PBS平衡至pH 8的蛋白A柱MabSelect(Amersham Biosciences#17-5199-02)。用5柱体积的pH 8的PBS洗涤柱。抗体用50 mM pH 3的柠檬酸盐-磷酸盐缓冲液洗脱。洗脱的抗体用1M pH 8的磷酸盐缓冲液中和。纯化的抗体用pH 7.4的PBS透析。使用鼠单克隆抗体标准曲线通过SDS-PAGE确定抗体浓度。

[0603] 使用Immunopure Fab试剂盒(Pierce#44885)通过完整抗体的木瓜蛋白酶蛋白水解来制备Fab,并按照制造商的说明书通过流过蛋白A色谱来纯化。使用已知浓度的标准Fab(通过氨基酸分析确定)通过ELISA和 /或SDS-PAGE电泳以及通过使用1 OD=0.6mg/ml(或基于氨基酸序列的理论等价物)的A280确定浓度。

[0604] Fab的亲和力确定。使用BIACORE3000™表面等离子体共振(SPR) 系统(Biacore,INC,Piscataway NJ)并利用制造商自己的运行缓冲液 HBS-EP(10mM HEPES pH 7.4,150mM NaCl,3mM EDTA,0.005%v/v 聚山梨酯P20)在25℃或37℃下确定抗CGRP单克隆抗体的亲和力。通过用SA芯片上预先固定的链霉亲和素捕获N末端生物素化CGRP肽(定制自GenScript Corporation,New Jersey或Global Peptide Services, Colorado)并测量在CGRP表面上

滴定的抗体Fab的结合动力学来确定亲和力。将生物素化CGRP稀释到HBS-EP中并以小于0.001mg/ml的浓度注射到芯片上。使用通过各个芯片通道的可变流动时间，获得了两个抗原密度范围：用于详细的动力学研究的＜50个响应单位(RU)，用于浓度研究和筛选的约800RU。以100μL/min注射纯化的Fab片段的通常跨1μM -0.1nM浓度的二倍或三倍系列稀释液(旨在为0.1-10x的估计K_D)1分钟，并允许10分钟的解离时间。在每个结合循环后，用25％v/v乙醇中的25mM NaOH再生表面，其耐受数百次循环。通过使用BIAevaluation 程序将数据拟合至1:1朗缪尔结合模型(Karlsson,R.Roos,H.Fagerstam, L.Petersson,B.(1994).Methods Enzymology 6.99-110)来同时获得动力学缔合速率(k_{on})和解离速率(k_{off})。全局平衡解离常数(K_D)或“亲和力”由比例$K_D=k_{off}/k_{on}$计算。鼠Fab片段的亲和力在表2和3中示出。

[0605] 鼠抗CGRP抗体的表位作图。为了确定抗CGRP抗体在人α-CGRP 上结合的表位，如上所述通过在SA传感器芯片上捕获N末端生物素化 CGRP片段第19-37位氨基酸和第25-37位氨基酸来测量Fab片段对多种 CGRP片段的结合亲和力。图1示出了在25℃下测量的其结合亲和力。如图1中所示，除抗体4901之外，所有抗体以与它们对全长人α-CGRP(1-37) 的结合亲和力相似的亲和力与人α-CGRP片段19-37和25-37结合。主要由于解离速率的损失，抗体4901以与全长人α-CGRP片段结合的亲和力相比六倍低的亲和力与人α-CGRP片段25-37结合。数据表明这些抗CGRP 抗体通常与CGRP的C末端结合。

[0606] 进行丙氨酸扫描以进一步表征人α-CGRP中参与抗CGRP抗体结合的的氨基酸。通过肽合成产生具有单丙氨酸替换的人α-CGRP的不同变体。它们的氨基酸序列与Biacore分析中使用的所有其他肽一起在表4中示出。如上所述使用Biacore确定抗CGRP抗体的Fab片段与这些变体的亲和力。如图1中所示，所有12种抗体均靶向C-末端表位，其中氨基酸F37是最关键的残基。F37突变成丙氨酸显著降低了亲和力或者甚至完全敲除了抗 CGRP抗体与肽的结合。接下来最重要的氨基酸残基是G33，然而，仅高亲和力抗体(7E9、8B6、10A8和7D11)受到该位置丙氨酸替换的影响。氨基酸残基S34在这四种高亲和力抗体的结合中也发挥显著但较小的作用。

[0607] 表2.抗CGRP单克隆抗体与人α-CGRP结合的特征及其拮抗剂活性

[0608]

抗体	25℃下与人α-CGRP的K_D（nM）	37℃下与人α-CGRP的K_D（nM）	25℃下基于细胞阻断人α-CGRP与其受体的结合（通过cAMP活化测量）	在放射性配体结合测定中测量的25℃（室温）下的IC_{50}（nM结合位点）。
7E9	1.0	0.9	是	2.5
8B6	1.1	1.2	是	4.0
10A8	2.1	3.0	是	n.d.
7D11	4.4	5.4	是	n.d.
6H2	9.3	42	是	12.9
4901	61	139	是	58
14E10	80	179	是	n.d.
9B8	85	183	否	n.d.
13C2	94	379	否	n.d.
14A9	148	581	否	n.d.
6D5	210	647	否	n.d.
1C5	296	652	否	n.d.

[0609] 注意：抗体4901是市售的(Sigma，产品号C7113)。

[0610] n.d.＝未确定

[0611] 表3.抗CGRP单克隆抗体与大鼠α-CGRP结合的特征及其拮抗剂活性

[0612]

抗体	37℃下与大鼠α-CGRP的K_D（nM）	25℃下基于细胞阻断大鼠α-CGRP与其受体的结合（通过cAMP活化测量）	隐神经测定中的体内阻断
4901	3.4	是	是
7E9	47	是	是
6H2	54	否	否
8B6	75	是	是
7D11	218	是	是
10A8	451	否	n.d.
9B8	876	否	n.d.
14E10	922	否	n.d.
13C2	> 1000	否	n.d.
14A9	> 1000	否	n.d.
6D5	> 1000	否	n.d.
1C5	> 1000	否	n.d.

[0613] “n.d.”表示没有对抗体进行测试。

[0614] 表4.人α-CGRP片段(SEQ ID NO:15-40)和相关肽(SEQ ID NO:41-47)的氨基酸序列。除了SEQ ID NO:36-40以外,所有肽都是C-末端酰胺化的。粗体残基表示点突变。

[0615]

CGRP	氨基酸序列	SEQ ID NO
1-37 (WT)	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	15
8-37	VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	16
19-37	SGGVVKNNFVPTNVGSKAF	17
P29A (19-37)	SGGVVKNNFVATNVGSKAF	18
K35A (19-37)	SGGVVKNNFVPTNVGSAAF	19
K35E (19-37)	SGGVVKNNFVPTNVGSEAF	20
K35M (19-37)	SGGVVKNNFVPTNVGSMAF	21
K35Q (19-37)	SGGVVKNNFVPTNVGSQAF	22
F37A (19-37)	SGGVVKNNFVPTNVGSKAA	23
25-38A	NNFVPTNVGSKAFA	24
25-37	NNFVPTNVGSKAF	25
F27A (25-37)	NNAVPTNVGSKAF	26
V28A (25-37)	NNFAPTNVGSKAF	27
P29A (25-37)	NNFVATNVGSKAF	28
T30A (25-37)	NNFVPANVGSKAF	29
N31A (25-37)	NNFVPTAVGSKAF	30
V32A (25-37)	NNFVPTNAGSKAF	31
G33A (25-37)	NNFVPTNVASKAF	32
S34A (25-37)	NNFVPTNVGAKAF	33
F37A (25-37)	NNFVPTNVGSKAA	34
26-37	NFVPTNVGSKAF	35
19-37-COOH	SGGVVKNNFVPTNVGSKAF	36
19-36-COOH	SGGVVKNNFVPTNVGSKA	37
1-36-COOH	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKA	38
1-19-COOH	ACDTATCVTHRLAGLLSRS	39
1-13-COOH	ACDTATCVTHRLA	40
大鼠α(1-37)	SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSEAF	41
大鼠α(19-37)	SGGVVKDNFVPTNVGSEAF	42
人β(1-37)	ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF	43
大鼠β(1-37)	SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSKAF	44
人降钙素(1-32)	CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP	45
人胰淀素(1-37)	KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY	46
人肾上腺髓质素(1-52)	YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDK DKDNVAPRSKISPQGY	47

[0616] 实施例2:使用体外测定筛选抗CGRP拮抗剂抗体

[0617] 使用基于细胞的cAMP活化测定和结合测定进一步筛选鼠抗CGRP 抗体的体外拮抗剂活性。

[0618] 通过cAMP测定来测量拮抗剂活性。在存在或不存在抗CGRP抗体 (终浓度为1-3000nM)或大鼠α-CGRP或人α-CGRP(终浓度为0.1 nM-10μM;作为c-AMP活化的阳性对照)的情况下,将5微升人或大鼠α-CGRP(终浓度为50nM))分配到384孔板(Nunc,目录号264657)中。将刺激缓冲液(20mM HEPES,pH 7.4,146mM NaCl,5mM KCl,1mM $CaCl_2$,1mM $MgCl_2$,以及500μM 3-异丁基-1-甲基黄嘌呤(IBMX))中的 10μl细胞(如果使用人α-CGRP则为人SK-N-MC;如果使用大鼠α-CGRP 则为来自ATCC的大鼠L6)添加到板的孔中。将板在室温下孵育30分钟。

[0619] 孵育后,使用HitHunter™酶片段互补测定(Applied Biosystems)按照制造商的说明书进行cAMP活化。该测定基于遗传改造的β-半乳糖苷酶,其由被称为酶接纳体(Enzyme Acceptor,EA)和酶供体(Enzyme Donor, ED)的两个片段组成。当两个片段分开时,酶无活性。当片段在一起时,它们可通过被称为互补的过程自发地重组以形成活性酶。EFC测定平台使用ED-cAMP肽缀合物,其中cAMP被抗cAMP识别。该ED片段能够与 EA重新缔合以形成活性酶。在该测定中,最佳地滴定抗cAMP抗体以结合ED-cAMP缀合物并抑制酶形成。细胞裂解物样品中的cAMP水平与 ED-cAMP缀合物竞争与抗cAMP抗体的结合。测定中游离ED缀合物的量与cAMP的浓度成比例。因此,通过活性酶的形成测量cAMP,所述活性酶的形成通过β-半乳糖苷酶发光底物的周转进行量化。通过添加10μl 裂解缓冲液和抗cAMP抗体(1:1比例)、随后在室温下孵育60分钟来进行cAMP活化测定。然后将10μl的ED-cAMP试剂添加到每个孔中并在室温下孵育60分钟。孵育后,将20μl EA试剂和CL混合物(含底物)(1:1 比例)添加到每个孔中并在室温下孵育1-3小时或过夜。用PMT仪器以1 秒/孔读取板,或用成像仪以30秒/位置读取板。在上表2和表3中鉴定了抑制α-CGRP活化cAMP的抗体(称为"是")。表2和3中的数据表明在测定中显示出拮抗剂活性的抗体通常具有高亲和力。例如,对于人α-CGRP 具有约80nM或更低K_D(在25℃下确定)或对大鼠α-CGRP具有约47nM 或更低K_D(在37℃下确定)的抗体在该测定中显示出拮抗剂活性。

[0620] 放射性配体结合测定。如先前的描述进行结合测定以测量抗CGRP 抗体阻断CGRP与受体结合的IC_{50}。Zimmermann等,Peptides 16:421-4, 1995;Mallee等,J.Biol.Chem.277:14294-8,2002。将来自SK-N-MC细胞的膜(25μg)在室温下在1mL总体积的含有10pM ^{125}I-人α-CGRP的孵育缓冲液(50mM Tris-HCl,pH 7.4,5mM $MgCl_2$,0.1%BSA)中孵育90分钟。为了确定抑制浓度(IC_{50}),将来自100倍储液的抗体或未标记的CGRP (作为对照)以不同的浓度溶解于孵育缓冲液中,并与膜和10pM ^{125}I-人α-CGRP同时孵育。通过已用0.5%聚乙烯亚胺封闭的玻璃微纤维过滤器 (GF/B,1μm)过滤终止孵育。绘制剂量响应曲线并使用以下等式确定 K_i值:$K_i = IC_{50}/(1+([配体]/K_D)$;其中人α-CGRP与存在于SK-N-MC细胞中的CGRP1受体的平衡解离常数KD=8pM,并且Bmax=0.025pmol/mg 蛋白质。将报道的IC_{50}值(就IgG分子而言)转化为结合位点(通过将其乘以2),以便可将其与通过Biacore确定的亲和力(K_D)进行比较(见表 2)。

[0621] 表2示出了鼠抗体7E9、8B6、6H2和4901的IC_{50}。数据表明抗体亲和力通常与IC_{50}相关联:具有较高亲和力(较低K_D值)的抗体在放射性配体结合测定中具有较低IC_{50}。

[0622] 实施例3:抗CGRP拮抗剂抗体对通过刺激大鼠隐神经诱导的皮肤血管扩张的影响

[0623] 为了测试抗CGRP抗体的拮抗剂活性,使用先前描述的大鼠模型测试抗体对通过刺激大鼠隐神经的皮肤血管扩张的影响。Escott等,Br.J. Pharmacol.110:772-776,1993。在该大鼠模型中,隐神经的电刺激诱导从神经末梢释放CGRP,导致皮肤血流提高。在隐神经刺激之后测量雄性 Sprague Dawley大鼠(170-300g,来自Charles River Hollister)的足部皮肤中的血流。用2%异氟烷将大鼠维持在麻醉下。在实验开始时给予托西溴苄铵(30mg/kg,i.v.施用),以使由于隐神经交感神经纤维的伴随刺激引起的血管收缩最小化。通过使用恒温连接至温控加热垫的直肠探针将体温保持在37℃。除了测试化合物和对照通过尾静脉注射的图3中所示的实验,以及其中抗体4901和7D11腹膜内(IP)注射的图2A和2B中所示的实验,通过右股静脉静脉内给予包括抗体、阳性对照(CGRP 8-37) 和载剂(PBS,0.01% Tween 20)的化合物。由于其半衰期短,阳性对照化合物CGRP 8-37(血管扩张拮抗剂)在神经刺激之前3-5分钟以400 nmol/kg(200μl)给予。Tan等,Clin.Sci.89:656-73,1995。抗体以不同剂量(1mg/kg、2.5mg/kg、5mg/kg、10mg/kg和25mg/kg)给予。

[0624] 对于图2A和2B中所示的实验,在电脉冲刺激之前72小时腹膜内(IP) 施用抗体4901(25mg/kg)、抗体7D11(25mg/kg)或载剂对照(具有0.01% Tween 20的PBS)。对于图3中所示的实验,在电脉冲刺激之前24小时静脉内施用抗体4901(1mg/kg、2.5mg/kg、5mg/kg或25mg/kg)或载剂对照(具有0.01%Tween 20的PBS)。在施用抗体或载剂对照之后,手术暴露右后肢的隐神经,近侧切开并用塑料包裹物覆盖以防止干燥。将激光多普勒探头置于后爪皮肤的背中部侧(medio-dorsal side),这是由隐神经支配的区域。使用激光多普勒流量计监测作为血细胞通量测量的皮肤血流。当稳定的基线通量(小于5%变化)建立至少5分钟时,将神经放置在铂双极电极上并用60个脉冲(2Hz,10V,1ms,持续30秒)进行电刺激并随后在20分钟后再次进行。对于电脉冲刺激的每个通量响应,通过通量- 时间曲线下的面积(AUC,其等于通量变化乘以时间变化)评估皮肤血流的累积变化。取对两次刺激的血流响应的平均值。将动物保持在麻醉下1 至3小时。

[0625] 如图2A和图2B所示,与对照相比,通过在隐神经上施加电脉冲刺激的血流提高被抗体CGRP 8-37(400nmol/kg,i.v.施用)、抗体4901(25 mg/kg,ip施用)或抗体7D11(25mg/kg,ip施用)的存在抑制。在隐神经刺激之前3-5分钟施用CGRP 8-37;并且在隐神经刺激之前72小时施用抗体。如图3所示,通过在隐神经上施加电脉冲刺激的血流提高被在隐神经刺激之前24小时静脉内施用的不同剂量(1mg/kg、2.5mg/kg、5mg/kg 和25mg/kg)抗体4901的存在抑制。

[0626] 对于图4A和4B中所示的实验,在抗体施用之前手术暴露隐神经。通过手术暴露右后肢的隐神经,近侧切开并用塑料包裹物覆盖以防止干燥。将激光多普勒探头置于后爪皮肤的背中部侧,这是由隐神经支配的区域。使用激光多普勒流量计监洲作为血细胞通量测量的皮肤血流。托西溴苄铵注射之后30至45分钟,当稳定的基线通量(小于5%变化)建立至少5分钟时,将神经放置在铂双极电极上并进行电刺激(2Hz,10V,1ms,持续30秒)并在20分钟后再次进行。使用对两次刺激的血流通量响应的平均值来建立对电刺激的基线响应(时间0)。随后静脉内(i.v.)施用抗体4901(1mg/kg或10mg/kg)、抗体7E9(10mg/kg)、抗体8B6(10mg/kg) 或载剂(具有0.01%Tween 20的PBS)。随后在抗体或载剂施用之后30 分钟、60分钟、90分钟和120分钟对神经进行刺激(2Hz,10V,1ms,持续30秒)。将动物保持在麻醉下约

3小时的时间。对于电脉冲刺激的每个通量响应，通过通量-时间曲线下面积(AUC，其等于通量变化乘以时间变化)评估皮肤血流的累积变化。

[0627] 如图4A所示，当在抗体施用之后60分钟、90分钟和120分钟施加电脉冲刺激时，通过在隐神经上施加电脉冲刺激的血流提高被1mg/kg i.v. 施用的抗体4901的存在显著抑制，并且当在抗体施用之后30分钟、60 分钟、90分钟和120分钟施加电脉冲刺激时，通过在隐神经上施加电脉冲刺激的血流提高被10mg/kg i.v.施用的抗体4901的存在显著抑制。图4B表明当在抗体施用之后30分钟、60分钟、90分钟和120分钟施加电脉冲刺激时，通过在隐神经上施加电脉冲刺激的血流提高被抗体7E9(10 mg/kg，i.v.施用)的存在显著抑制，并且当在抗体施用之后30分钟施加电脉冲刺激时，被抗体8B6(10mg/kg，i.v.施用)的存在显著抑制。

[0628] 这些数据表明抗体4901、7E9、7D11和8B6在阻断CGRP活性方面是有效的，如通过刺激大鼠隐神经诱导的皮肤血管扩张测量的。

[0629] 实施例4.抗CGRP抗体G1及其变体的表征

[0630] 抗CGRP抗体G1的重链可变区和轻链可变区的氨基酸序列在图5中示出。以下方法用于抗体G1及其变体的表达和表征。

[0631] 使用的表达载体。类似于Barbas(2001)Phage display:a laboratory manual，Cold Spring Harbor,NY,Cold Spring Harbor Laboratory Press pg. 2.10.Vector pComb3X)中的描述，抗体的Fab片段的表达在IPTG诱导型 lacZ启动子的控制下，然而，修改包括添加和表达以下额外结构域：人κ轻链恒定结构域和IgG2人免疫球蛋白的CH1恒定结构域，Igγ-2链C区，蛋白质登录号P01859;免疫球蛋白κ轻链(人(Homo sapiens))，蛋白质登录号CAA09181。

[0632] 小规模Fab制备。从用Fab文库转化的大肠杆菌(使用电穿孔感受态 TG1细胞或化学感受态Top 10细胞)，将单菌落用于接种主板(琼脂LB+ 羧苄青霉素(50μg/mL)+2％葡萄糖)和工作板(2mL/孔，96孔/板)，其中每个孔含有1.5mL LB+羧苄青霉素(50μg/mL)+2％葡萄糖。将透气黏合密封件(ABgene，Surrey，UK)施加至板。将两个板在30℃下孵育 12-16小时；工作板剧烈摇动。将主板储存在4℃直至需要，而将来自工作板的细胞沉淀(4000rpm，4℃，20分钟)并重悬于1.0mL LB+羧苄青霉素(50μg/mL)+0.5mM IPTG中，以通过在30℃剧烈摇动5小时来诱导Fab的表达。将诱导的细胞在4000rpm、4℃下离心20分钟，并重悬于 0.6mL Biacore HB-SEP缓冲液(10mM HEPES pH 7.4，150mM NaCl，3 mM EDTA，0.005％v/v P20)中。通过冷冻(-80℃)并随后在37℃解冻完成HB-SEP重悬细胞的裂解。将细胞裂解物在4000rpm、4℃下离心1小时以将碎片从含有Fab的上清液中分离，其随后使用Millipore MultiScreen Assay System 96孔过滤板和真空分流管过滤(0.2μm)。使用Biacore通过将经过滤的上清液注射经过传感器芯片上的CGRP上来分析所述经过滤的上清液。从主板中收集表达Fab的经亲和力选择的克隆，其提供用于PCR、测序和质粒制备的模板DNA

[0633] 大规模Fab制备。为了获得动力学参数，如下以更大规模表达Fab。用来自亲和力选择的表达Fab的大肠杆菌克隆的1mL“起始”过夜培养物接种含有150mL LB+羧苄青霉素(50μg/mL)+2％葡萄糖的锥形瓶 (Erlenmeyer flask)。剩余的起始培养物(～3mL)用于制备质粒DNA (QIAprep mini-prep，Qiagen试剂盒)，用于测序和进一步操作。将大培养物在30℃剧烈摇动下孵育直至OD_{600nm}达到1.0(通常为12-16小时)。通过在4000rpm、4℃下离心20分钟

使细胞沉淀,并重悬于150mL LB+羧苄青霉素(50μg/mL)+0.5mM IPTG中。在30℃下表达5小时之后,通过在4000rpm、4℃下离心20分钟使细胞沉淀,重悬于10mL Biacore HBS-EP缓冲液中,并使用单次冷冻(-80℃)/解冻(37℃)循环裂解。通过在4000rpm、4℃下离心1小时使细胞裂解物沉淀,并收集上清液并过滤(0.2μm)。将经过滤的上清液加载到用pH 8的PBS平衡的Ni-NTA 超流琼脂糖凝胶(superflow sepharose)(Qiagen,Valencia,CA)柱上,然后用5个柱体积的pH 8的PBS洗涤。单独的Fab用PBS(pH 8)+300mM 咪唑在不同级分中洗脱。合并含有Fab的级分并在PBS中透析,然后在亲和力表征之前通过ELISA进行定量。

[0634] 完整抗体制备。为了表达完整抗体,将重和轻链可变区克隆到哺乳动物表达载体中,并使用lipofectamine转染到HEK293细胞中用于瞬时表达。使用标准方法使用蛋白A纯化抗体。

[0635] 载体pDb.CGRP.hFcGI是包含G1抗体重链的表达载体,并且适用于重链的瞬时表达或稳定表达。载体pDb.CGRP.hFcGI具有对应于以下区域的核苷酸序列:鼠巨细胞病毒启动子区(核苷酸7-612);合成内含子(核苷酸613-1679);DHFR编码区(核苷酸688-1253);人生长激素信号肽(核苷酸1899-1976);G1的重链可变区(核苷酸1977-2621);包含以下突变的人重链IgG2恒定区:A330P331至S330S331(参照野生型IgG2序列进行氨基酸编号;参见Eur.J.Immunol.(1999)29:2613-2624)。载体 pDb.CGRP.hFcGI于2005年7月15日保藏于ATCC,并被分配ATCC登记号PTA-6867。

[0636] 载体pEb.CGRP.hKGI是包含G1抗体轻链的表达载体,并且适合于轻链的瞬时表达。载体pEb.CGRP.hKGI具有对应于以下区域的核苷酸序列:鼠巨细胞病毒启动子区域(核苷酸2-613);人EF-1内含子(核苷酸 614-1149);人生长激素信号肽(核苷酸1160-1237);抗体G1轻链可变区(核苷酸1238-1558);人κ链恒定区(核苷酸1559-1882)。载体 pEb.CGRP.hKGI于2005年7月15日保藏于ATCC,并被分配ATCC登记号PTA-6866。

[0637] Biacore测定用于亲和力确定。使用BIACORE3000™表面等离子体共振(SPR)系统(Biacore,INC,Piscataway NJ)在25℃或37℃下确定G1 单克隆抗体及其变体的亲和力。通过经预先固定的链霉亲和素(SA传感器芯片)捕获N末端生物素化CGRP或片段并测量经过芯片上的CGRP 或片段而滴定的抗体G1 Fab片段或变体的结合动力学来确定亲和力。所有Biacore测定均在HBS-EP运行缓冲液(10mM HEPES pH 7.4,150mM NaCl,3mM EDTA,0.005% v/v聚山梨酯P20)中进行。通过在HBS-EP 缓冲液中将N-生物素化CGRP稀释至浓度小于0.001mg/mL的浓度并使用可变的接触时间将其注射经过SA传感器芯片来制备CGRP表面。对应于捕获水平<50个响应单位(response unit,RU)的低容量表面用于高分辨率动力学研究,而高容量表面(约800RU的捕获CGRP)用于浓度研究、筛选和溶液亲和力确定。通过以二或三倍增量将抗体G1 Fab连续稀释至跨越1uM-0.1nM(旨在为0.1-10×的估计K_D)的浓度来获得动力学数据。样品通常以100μL/min注射1分钟,并且允许至少10分钟的解离时间。在每个结合循环之后,用25%v/v乙醇中的25mM NaOH使表面再生,其耐受超过数百次循环。使用BIAevaluation程序将整个滴定系列(通常一式两份地产生)拟合至1:1朗缪尔结合模型。这为每种结合相互作用返回了独特的缔合和解离动力学速率常数(分别为k_{on}和k_{off}),其比值给出了平衡解离常数($K_D=k_{off}/k_{on}$)。以这种方式确定的亲和力(K_D值)表 6和7中列出。

[0638] 具有极慢解离速率的结合相互作用的高分辨率分析。对于具有极慢解离速率的相互作用(特别地,在25℃下抗体G1 Fab与芯片上的人α-CGRP 结合),在两部分实验中获得亲

和力。使用具有以下修改的上述方案。通过以100μL/分钟注射跨550nM-1nM的2倍滴定系列(一式两份)30秒并允许仅30秒解离阶段来确定缔合速率常数(k_{on})。通过一式两份注射三种浓度(高、中和低)的相同滴定系列30秒并允许2小时解离阶段来确定解离速率常数(k_{off})。如表5中所示,通过将在这两种类型的实验中获得的k_{on}和k_{off}值组合获得每种相互作用的亲和力(K_D)。

[0639] 通过Biacore确定溶液亲和力。通过Biacore在37℃测量抗体G1对大鼠α-CGRP和F37A(19-37)人α-CGRP的溶液亲和力。使用高容量CGRP 芯片表面(选择高亲和力人α-CGRP用于检测目的),并且以5μL/min流动HBS-EP运行缓冲液。用3倍连续稀释中跨越1nM至1μM的终浓度的竞争性肽(大鼠α-CGRP或F37A(19-37)人α-CGRP)预孵育5nM恒定浓度(旨在等于或低于基于溶液的相互作用的预期K_D)的抗体G1 Fab 片段。在不存在或存在基于溶液的竞争性肽的情况下,将抗体G1 Fab溶液注射经过芯片上的CGRP并监测由于溶液竞争而造成的在芯片表面检测到的结合响应的消耗。使用校准曲线将这些结合响应转化为"游离Fab 浓度",所述校准曲线通过经过在芯片上的CGRP单独滴定抗体G1 Fab(5、2.5、1.25、0.625、0.325和0nM)来构建。将"游离Fab浓度"相对于用于产生每个数据点的竞争性基于溶液之肽的浓度作图,并使用BIAevaluation 软件拟合至溶液亲和力模型。以这种方式(间接)确定的溶液亲和力在表 5和7中示出,并且用于验证经过在SA芯片的N-生物素化CGRP直接注射Fab时获得的亲和力。由这两种方法确定的亲和力之间的密切相符证实,将N-生物素化形式的CGRP与芯片拴系(tether)不改变其天然溶液结合活性。

[0640] 下表5示出了通过使Fab片段流经SA芯片上的N-生物素化CGRP,通过Biacore确定抗体G1与人α-CGRP、人β-CGRP、大鼠α-CGRP和大鼠β-CGRP的结合亲和力。为了更好地解析具有极低解离速率的结合相互作用的亲和力,还在与该测定方向互补的两部分实验中确定了亲和力,还确定了大鼠α-CGRP相互作用的溶液亲和力(如上所述)。在这两种测定方向上测量的亲和力的密切相符证实,在N-生物素化并拴系于SA芯片时,天然大鼠α-CGRP在溶液中的结合亲和力没有改变。

[0641] 表5.在芯片上的CGRP上滴定的抗体G1 Fab的结合亲和力

[0642]

芯片上的CGRP	温度(℃)	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
人α-CGRP	25	1.86×10^5	7.80×10^{-6}	0.042 (7%, n=4)*
人α-CGRP	37	5.78×10^5	3.63×10^{-5}	0.063 (4%, n=2)*
人β-CGRP	37	4.51×10^5	6.98×10^{-5}	0.155
大鼠α-CGRP	25	5.08×10^4	6.18×10^{-5}	1.22 (12%, n=2)*
大鼠α-CGRP	37	1.55×10^5	3.99×10^{-4}	2.57* (溶液 K_D=10 (50%, n=4)**
大鼠β-CGRP	37	5.16×10^5	7.85×10^{-5}	0.152

[0643] *在高分辨率两部分实验中确定α-CGRP(大鼠和人)的亲和力,其中监测解离阶段2小时 (k_{on}、k_{off}和K_D的值表示一式n份重复实验的平均值和表示为变异百分比的标准差)。β-CGRP (大鼠和人)的亲和力通过使用仅20分钟解离阶段的全局分析来确定,所述解离阶段的准确度不足以量化它们的极端解离速率(它们的解离速率可能比此处所述的更慢,并因此它们的亲和力可能甚至更高)。抗体G1 Fab从所有CGRP(除了α-大鼠CGRP)解离得极其缓慢,解离速率接近Biacore测定的分辨极限(尤其是在25℃)。

[0644] **通过测量在用基于溶液的大鼠α-CGRP竞争剂预孵育的抗体G1 Fab在芯片上的CGRP处检测到的结合响应的消耗来确定溶液亲和力。

[0645] 下表6示出了与抗体G1相比具有氨基酸序列变异的抗体及其对大鼠α-CGRP和人α-CGRP二者的亲和力。相对于G1的序列描述了表6中所示变体的所有氨基酸替换。Fab片段的结合亲和力通过Biacore通过使其流经SA芯片上的CGRP来确定。

[0646] 表6.通过Biacore在37℃下确定的抗体G1变体的氨基酸序列和结合亲和力数据。

[0647]

克隆	L1	L2	H2	HC-FW3	α-大鼠 k_{off} (1/s)	α-大鼠 K_D (nM)	α-人 k_{off} (1/s)	α-人 K_D (nM)
G1					$3.99x10^{-4}$	2.57	$3.63 x10^{-5}$	0.063
M1				A100L	$1.10x10^{-3}$		$1.73x10^{-4}$	
M2				L99A A100R	$2.6x10^{-3}$	58	$3.1x10^{-4}$	3
M3				L99A A100S	$2.0x10^{-3}$	61	$2.1x10^{-4}$	1.7
M4				L99A A100V	$1.52x10^{-3}$	84.4	$6.95x10^{-5}$	0.43
M5				L99A A100Y	$7.35x10^{-4}$	40.8	$3.22x10^{-5}$	0.20
M6				L99N	$7.84x10^{-4}$	43.6	$1.33x10^{-4}$	0.83
M7				L99N A100C	$9.18x10^{-4}$	51.0	$2.43x10^{-4}$	1.52
M8				L99N A100G	$7.45x10^{-4}$	41.4	$9.20x10^{-5}$	0.58
M9				L99N A100Y	n.d.	n.d.	$1.00x10^{-5}$	0.06
M10				L99S A100S	$1.51x10^{-3}$	83.9	$1.73x10^{-4}$	1.08
M11				L99S A100T	$4.83x10^{-3}$	268.3	$2.83x10^{-4}$	1.77
M12				L99S A100V	$1.94x10^{-3}$	107.8	$1.01x10^{-4}$	0.63
M13				L99T A100G	$1.84x10^{-3}$	102.2	$1.86x10^{-4}$	1.16
M14				L99T A100K	n.d.	n.d.	$1.00x10^{-5}$	0.06
M15				L99T A100P	$1.15x10^{-3}$	63.9	$1.58x10^{-5}$	0.10

[0648]

克隆	L1	L2	H2	HC-FW3	α-大鼠 k_{off} (1/s)	α-大鼠 K_D (nM)	α-人 k_{off} (1/s)	α-人 K_D (nM)
M16				L99T A100S	$9.96x10^{-4}$	55.3	$1.65x10^{-4}$	1.03
M17				L99T A100V	$2.06x10^{-3}$	114.4	$1.85x10^{-4}$	1.16
M18				L99V A100G	$1.22x10^{-3}$	67.8	$7.03x10^{-5}$	0.44
M19				L99V A100R	n.d.	n.d.	$1.00x10^{-5}$	0.06
M20	R28W			L99R A100L	$1.44x10^{-3}$	80.0	$1.36x10^{-4}$	0.85
M21	R28W			L99S	$6.95x10^{-4}$	15.2	$1.42x10^{-4}$	1.23
M22	R28W			L99T	$1.10x10^{-3}$	61.1	$1.16x10^{-4}$	0.73
M23	R28G			L99T A100V	$7.99x10^{-4}$	44.4	$1.30x10^{-4}$	0.81
M24	R28L			L99T A100V	$1.04x10^{-3}$	57.8	$1.48x10^{-4}$	0.93
M25	R28N			L99T A100V	$1.4x10^{-3}$	76	$1.4x10^{-4}$	1.3
M26	R28N		A57G	L99T A100V	$9.24x10^{-4}$	51.3	$1.48x10^{-4}$	0.93
M27	R28N T30A			L99T A100V	$3.41x10^{-3}$	189.4	$3.57x10^{-4}$	2.23
M28	R28N T30D		E54R A57N	L99T A100V	$1.25x10^{-3}$	69.4	$9.96x10^{-5}$	0.62
M29	R28N T30G			L99T A100V	$3.59x10^{-3}$	199.4	$3.80x10^{-4}$	2.38
M30	R28N T30G		E54K A57E	L99T A100V	$6.38x10^{-3}$	354.4	$5.90x10^{-4}$	3.69
M31	R28N T30G		E54K A57G	L99T A100V	$3.61x10^{-3}$	200.6	$3.47x10^{-4}$	2.17
M32	R28N T30G		E54K A57H	L99T A100V	$2.96x10^{-3}$	164.4	$2.71x10^{-4}$	1.69
M33	R28N T30G		E54K A57N S58G	L99T A100V	$9.22x10^{-3}$	512.2	$7.50x10^{-4}$	4.69
M34	R28N T30G		E54K A57N S58T	L99T A100V	$2.17x10^{-3}$	120.6	$6.46x10^{-4}$	4.04
M35	R28N T30G		E54K A57S	L99T A100V	$3.99x10^{-3}$	221.7	$3.39x10^{-4}$	2.12
M36	R28N T30R			L99T A100V	$4.79x10^{-3}$	266.1	$2.39x10^{-4}$	1.49
M37	R28N T30S		A57G	L99T A100V	$1.45x10^{-3}$	80.6	$2.26x10^{-4}$	1.41
M38	R28N T30W			L99T A100V	$5.11x10^{-3}$	283.9	$2.18x10^{-4}$	1.36
M39	R28N	G50A L56T	A57N S58Y	L99T A100V	$9.95x10^{-3}$	552.8	$4.25x10^{-4}$	2.66
M40	R28N	G50A L56T	E54K A57L	L99T A100V	0.36	20000.0	$1.28x10^{-3}$	8.00
M41	R28N	G50A	E54K	L99T	$4.53x10^{-3}$	251.7	$2.10x10^{-4}$	1.31

[0649]

克隆	L1	L2	H2	HC-FW3	α-大鼠 k_{off} (1/s)	α-大鼠 K_D (nM)	α-人 k_{off} (1/s)	α-人 K_D (nM)
		L56T	A57N E64D	A100V				
M42	R28N	G50A L56T	E54K A57N H61F	L99T A100V	$7.52x10^{-3}$	417.8	$4.17x10^{-4}$	2.61
M43	R28N	G50A L56T	E54K A57N S58C	L99T A100V	$4.53x10^{-3}$	251.7	$2.63x10^{-4}$	1.64
M44	R28N	G50A L56T	E54K A57N S58E	L99T A100V	$6.13x10^{-3}$	443	$2.10x10^{-4}$	2.05
M45	R28N	G50A L56T	E54K A57N S58E E64D	L99T A100V	$5.58x10^{-3}$	259	$2.11x10^{-4}$	1.85
M46	R28N	G50A L56T	E54K A57N S58E H61F	L99T A100V	$2.94x10^{-3}$	163.3	$5.39x10^{-4}$	3.37
M47	R28N	G50A L56T	E54K A57N S58G	L99T A100V	$8.23x10^{-3}$	457.2	$3.32x10^{-4}$	2.08
M48	R28N	G50A L56T	E54K A57N S58L	L99T A100V	0.0343	1905.6	$8.42x10^{-4}$	5.26
M49	R28N	G50A L56T	E54K A57N S58Y H61F	L99T A100V	0.0148	822.2	$5.95x10^{-4}$	3.72
M50	R28N	G50A L56T	E54K A57R	L99T A100V	$5.30x10^{-3}$	294.4	$4.06x10^{-4}$	2.54
M51	R28N	L56I	E54K A57G	L99T A100V	$1.18x10^{-3}$	65.6	$1.31x10^{-4}$	0.82
M52	R28N	L56I	E54K A57N S58A	L99T A100V	$2.29x10^{-3}$	127.2	$2.81x10^{-4}$	1.76
M53	R28N	L56I	E54K A57N S58G	L99T A100V	$1.91x10^{-3}$	106.1	$3.74x10^{-4}$	2.34
M54	R28N T30A	G50A	E54K A57N S58P	L99T A100V	$2.16x10^{-3}$	120.0	$1.79x10^{-3}$	11.19
M55	R28N T30A	L56S	E54K A57N S58E E64D	L99T A100V	$5.85x10^{-3}$	325.0	$4.78x10^{-4}$	2.99
M56	R28N T30D	L56S	E54K A57N H61F	L99T A100V	$9.35x10^{-3}$	519.4	$4.79x10^{-4}$	2.99
M57	R28N T30D	L56S	E54K A57N	L99T A100V	0.0104	1,200	$3.22x10^{-4}$	3.08

[0650]

克隆	L1	L2	H2	HC-FW3	α-大鼠 k_{off} (1/s)	α-大鼠 K_D (nM)	α-人 k_{off} (1/s)	α-人 K_D (nM)
			S58E					
M58	R28N T30D	L56S	E54K A57N S58I H61F	L99T A100V	无结合	n.d.	$1.95x10^{-3}$	12.19
M59	R28N T30D	L56S	E54K A57N S58N H61F	L99T A100V	0.0123	683.3	$5.24x10^{-4}$	3.28
M60	R28N T30D	L56S	E54K A57N S58R H61F	L99T A100V	0.0272	1511.1	$9.11x10^{-4}$	5.69
M61	R28N T30G	A51H	E54Q A57N H61F	L99T A100V	$5.21x10^{-3}$	289.4	$4.59x10^{-4}$	2.87
M62	R28N T30G	A51H L56T	E54K A57N S58E	L99T A100V	$5.75x10^{-3}$	242	$5.57x10^{-4}$	5.86
M63	R28N T30G	G50A	E54K A57N S58T	L99T A100V	$2.65x10^{-3}$	147.2	$1.50x10^{-3}$	9.38
M64	R28N T30G	G50A	E54K A57N S58V	L99T A100V	0.0234	1300.0	$1.32x10^{-3}$	8.25
M65	R28N T30G	G50A L56I	E54K A57C	L99T A100V	$4.07x10^{-3}$	226.1	$8.03x10^{-4}$	5.02
M66	R28N T30G	L56I	E54K A57E	L99T A100V	$5.11x10^{-3}$	283.9	$5.20x10^{-4}$	3.25
M67	R28N T30G	L56I	E54K A57F	L99T A100V	$1.71x10^{-3}$	95.0	$8.20x10^{-4}$	5.13
M68	R28N T30G	L56I	E54K A57N S58D E64D	L99T A100V	$6.76x10^{-3}$	375.6	$4.28x10^{-4}$	2.68
M69	R28N T30G	L56I	E54K A57N S58E	L99T A100V	$1.81x10^{-3}$	100.6	$7.33x10^{-4}$	4.58
M70	R28N T30G	L56I	E54K A57S	L99T A100V	$6.07x10^{-3}$	337.2	$5.59x10^{-4}$	3.49
M71	R28N T30G	L56I	E54K A57Y	L99T A100V	$2.12x10^{-3}$	117.8	$1.28x10^{-3}$	8.00
M72	R28N T30G	L56S	E54K	L99T A100V	$3.95x10^{-3}$	219.4	$4.00x10^{-4}$	2.50
M73	R28N T30G	L56S	E54K A57N S58Y E64D	L99T A100V	$3.00x10^{-3}$	166.7	$2.55x10^{-4}$	1.59
M74	R28N T30G	L56S	E54K A57S	L99T A100V	$6.03x10^{-3}$	335.0	$5.97x10^{-4}$	3.73
M75	R28N	L56S	E54K	L99T	$1.87x10^{-2}$	1038.9	$1.16x10^{-3}$	7.25

[0651]

克隆	L1	L2	H2	HC-FW3	α-大鼠 k_{off} (1/s)	α-大鼠 K_D (nM)	α-人 k_{off} (1/s)	α-人 K_D (nM)
	T30G		A57V	A100V				
M76	R28N T30S	G50A L56T	A57G	L99T A100V	$1.16x10^{-3}$	64.4	$3.64x10^{-4}$	2.28
M77	R28N T30S	G50A L56T	E54K A57D	L99T A100V	0.0143	794.4	$4.77x10^{-4}$	2.98
M78	R28N T30S	G50A L56T	E54K A57N S58T	L99T A100V	0.167	9277.8	$1.31x10^{-3}$	8.19
M79	R28N T30S	G50A L56T	E54K A57P	L99T A100V	0.19	10555.6	$1.29x10^{-3}$	8.06
M80	R28N T30S	L56I	E54K A57N S58V	L99T A100V	0.0993	5516.7	$2.09x10^{-3}$	13.06
M81	R28N T30S	L56S	E54K A57N S58E	L99T A100V	$4.29x10^{-3}$	238.3	$4.90x10^{-4}$	3.06
M82	R28N T30V	A51H L56T	A57N	L99T A100V	$6.99x10^{-3}$	388.3	$8.77x10^{-4}$	5.48
M83	R28N T30V	A51H L56T	E54K A57N S58M H61F	L99T A100V	无结合	n.d.	$9.33x10^{-4}$	5.83
M84	R28N T30V	A51H L56T	E54N A57N	L99T A100V	$1.76x10^{-2}$	977.8	$1.08x10^{-3}$	6.75

[0652] 所有CDR包括Kabat CDR和Chothia CDR二者。氨基酸残基按顺序编号(见图5)。所有克隆都具有与G1相同的L3+H1+H3序列。

[0653] $K_D = k_{off}/k_{on}$。除了具有下划线的那些,所有k_{off}值均在筛选模式中确定,其通过Fab浓度系列的全局分析(以高分辨率模式分析G1)获得。因此,具有下划线的K_D值通过测量k_{on}经实验确定。其他k_{on}值估计与M25相同。

[0654] n.d.=未确定

[0655] 为了确定人α-CGRP上被抗体G1识别的表位,使用上述Biacore测定。人α-CGRP作为N-生物素化形式购买,以能够通过SA传感器芯片进行其高亲和力捕获。确定在不存在或存在CGRP肽的情况下G1 Fab片段与芯片上的人α-CGRP的结合。通常来说,注射2000:1mol肽/Fab溶液(例如,50nM G1 Fab中的10μM肽)经过芯片上的人α-CGRP。图6示出了竞争肽阻断的结合百分比。图6中示出的数据表明阻断G1 Fab与人α-CGRP 100%结合的肽是人α-CGRP的1-37(WT)、8-37、26-37、P29A (19-37)、K35A(19-37)、K35E(19-37)和K35M(19-37);β-CGRP(WT) 的1-37;大鼠α-CGRP(WT)的1-37;以及大鼠β-CGRP(WT)的1-37。所有这些肽都在C-末端酰胺化。人α-CGRP的肽F37A(19-37)和19-37 (后者未在C-末端酰胺化)也阻断了G1 Fab与人α-CGRP的约80%-90%的结合。人α-CGRP的肽1-36(未在C-末端酰胺化)阻断G1 Fab与人α-CGRP的约40%的结合。人α-CGRP的肽片段19-36(在C-末端酰胺化)、人α-CGRP的肽片段1-13和1-19(均未在C-末端酰胺化)、以及人胰淀素、降钙素和肾上腺髓质素(全部在C-末端酰胺化)不竞争G1 Fab与人芯片上α-CGRP的结合。这些数据表明G1靶向CGRP的C-末端表位,并且最末端残基(F37)的身份及其酰胺化对于结合是重要的。

[0656] 还确定了G1 Fab与人α-CGRP变体的结合亲和力(在37℃)。下表7 示出了通过经过在芯片上的N-生物素化的人α-CGRP及变体滴定G1 Fab 直接测量的亲和力。表7中的数据表明抗体G1与C-末端表位结合,F37 和G33是最重要的残基。当在C-末端(其被酰胺化)添加额

外的氨基酸残基(丙氨酸)时,G1不与CGRP结合。

[0657] 表7.在37℃下测量的G1Fab与人α-CGRP及变体的结合亲和力(它们的氨基酸序列见表4)

[0658]

芯片上的CGRP	k_{on}(1/Ms)	k_{off}(1/s)	K_D(nM)
1-37(WT)	$4.68x10^5$	$7.63x10^{-5}$	0.16(高分辨率K_D=0.06)
19-37	$4.60x10^5$	$7.30x10^{-5}$	0.16
25-37	$3.10x10^5$	$8.80x10^{-5}$	0.28
F27A(25-37)	$3.25x10^5$	$1.24x10^{-4}$	0.38
V28A(25-37)	$3.32x10^5$	$9.38x10^{-5}$	0.28
P29A(25-37)	$2.26x10^5$	$1.78x10^{-4}$	0.79
T30A(25-37)	$1.79x10^5$	$8.41x10^{-5}$	0.47
N31A(25-37)	$2.17x10^5$	$1.14x10^{-4}$	0.53
V32A(25-37)	$2.02x10^5$	$3.46x10^{-4}$	1.71
G33A(25-37)	$2.07x10^5$	0.0291	141
S34A(25-37)	$2.51x10^5$	$7.64x10^{-4}$	3.04

[0659]

芯片上的CGRP	k_{on}(1/Ms)	k_{off}(1/s)	K_D(nM)
K35A(19-37)	$2.23x10^5$	$2.97x10^{-4}$	1.33
K35E(19-37)	$5.95x10^4$	$5.79x10^{-4}$	9.73
K35M(19-37)	$2.63x10^5$	$1.34x10^{-4}$	0.51
K35Q(19-37)	$1.95x10^5$	$2.70x10^{-4}$	1.38
F37A(25-37)	$8.90x10^4$	$8.48x10^{-3}$	95(溶液K_D=172nM)
38A(25-38A)	-	-	未检测到结合

[0660] 上述数据表明抗体G1结合的表位位于人α-CGRP的C末端,且人α-CGRP上的第33和37位氨基酸对于抗体G1的结合是重要的。此外,残基F37的酰胺化对于结合是重要的。

[0661] 实施例5:抗CGRP拮抗剂抗体G1对通过刺激大鼠隐神经诱导的皮肤血管扩张的影响

[0662] 为了测试抗CGRP抗体G1的拮抗剂活性,使用实施例3中描述的大鼠模型测试抗体对通过刺激大鼠隐神经的皮肤血管扩张的影响。简言之,用2%异氟烷将大鼠维持在麻醉下。在实验开始时给予托西溴苄铵(30 mg/kg,i.v.施用),以使由于隐神经交感神经纤维的伴随刺激引起的血管收缩最小化。通过使用恒温连接至温控加热垫的直肠探针将体温保持在 37℃。通过手术暴露右后肢的隐神经,近侧切开并用塑料包裹物覆盖以防止干燥。将激光多普勒探头置于后爪皮肤的背中部侧,这是由隐神经支配的区域。使用激光多普勒流量计监测作为血细胞通量测量的皮肤血流。在用甲苯磺酸苄酯注射后三十至四十五分钟确定注射的两小时内抗体的作用的实验中,当稳定的基线通量(小于5%变化)建立至少5分钟时,将神经放置在铂双极电极上并进行电刺激(2Hz,10V,1ms,持续30秒)并在20分钟后再次进行。使用对这两次刺激的血流通量响应的平均值来建立对电刺激的基线响应(时间0)。然后静脉内(i.v.)施用抗体G1(1mg/kg 或10mg/kg)或载剂(与10mg/kg G1等体积的具有

0.01％Tween 20的 PBS)。随后在抗体施用之后30分钟、60分钟、90分钟和120分钟对神经进行刺激(2Hz,10V,1ms,持续30秒)。将动物保持在麻醉下约3小时。对于电脉冲刺激的每个通量响应,通过通量-时间曲线下面积(AUC,其等于通量变化乘以时间变化)评估皮肤血流的累积变化。

[0663] 如图7中所示,当在抗体施用之后90分钟电刺激隐神经时,与载剂相比,通过在隐神经上施加电脉冲刺激的血流提高被1mg/kg(i.v.施用) 抗体G1的存在显著抑制。当在抗体施用之后90分钟和120分钟电刺激隐神经时,与载剂相比,通过在隐神经上施加电脉冲刺激的血流提高被10 mg/kg(i.v.施用)抗体G1的存在显著抑制。

[0664] 在隐神经测定中在较长时间点确定抗体效应的实验中,在将动物准备用于如上所述隐神经刺激之前24小时或7天,向大鼠i.v.注射指示剂量的抗体。在这些实验中,在给药前不能建立个体大鼠对电脉冲刺激的基线响应,因此在24小时或7天将经处理组与用载剂(PBS,0.01％Tween 20) 给药的动物进行比较。

[0665] 如图8A和8B所示,与相同时间点给药的载剂组相比,在刺激之前 24小时或7天以10mg/kg或3mg/kg的G1给药的动物组中,通过隐神经刺激诱发的背内侧后爪皮肤中的血流提高被显著抑制。

[0666] 图8C表示应用于图8A和8B中所示剂量响应数据以确定50％最大效应所需剂量(EC_{50})的曲线拟合分析。24小时的EC_{50}是1.3mg/kg,且7 天时的EC_{50}略低(0.8mg/kg)。

[0667] 实施例6:硬脑膜动脉(闭合颅骨窗口)测定中抗CGRP拮抗剂抗体mu7E9的急性作用

[0668] 闭合颅骨窗口模型(Closed Cranial Window Model):本实验的目的是确定抗CGRP拮抗剂抗体的急性作用并将其与CGRP受体拮抗剂 BIBN4096BS的急性作用相比较。如先前所述进行实验(Williamson等, Cephalalgia 17(4):518-24(1997)),并进行了以下修改。将Sprague Dawley 大鼠(300-400g)用70mg/kg i.p.戊巴比妥麻醉。用20mg/kg/hr i.v.戊巴比妥维持麻醉。将大鼠通过颈静脉插管用于递送所有药物。用穿过股动脉进入腹主动脉的探针(mikro-tip导管,Millar Instruments)监测血压。对大鼠进行气管切开,并将呼吸频率维持在每分钟75次呼吸,体积为3.5mL。在将头部固定在立体定向器械中并除去头皮之后,通过用牙钻使骨头变薄来制作正好在矢状缝外侧的左顶骨区域的2×6mm窗口。使用显微操作器,将铂双极电极下降到表面上并用重矿物油覆盖。在电极窗的外侧产生另一个5×6mm的窗口,并填充重矿物油,通过它用CCD照相机和视频尺寸分析仪(Living Systems)连续监测中脑膜动脉(middle meningeal artery, MMA)分支的直径。制备后,使大鼠休息不少于45分钟。建立对电刺激的基线响应(115V,10hz,0.5ms脉冲,30秒),并随后向大鼠i.v.施用实验化合物(10mg/kg mu7E9、300μg/kg BIBN4096BS、或PBS 0.01％,Tween 20)。在施用之后5(BIBN4096BS)、30、60、90和120分钟进行另外的电刺激。所有数据均使用图表软件(ADInstruments)记录。

[0669] 如图9所示,10mg/kg的mu7E9在给药之后60分钟内显著阻断通过电场刺激引起的MMA扩张,并在整个测定期间(120分钟)保持该作用。为了比较,BIBN4096BS在给药的5分钟内阻断MMA扩张,但到90分钟时该作用已经完全消失。BIBN4096BS与mu7E9之间的阻断量级相当。

[0670] 实施例7:硬脑膜动脉(闭合颅骨窗口)测定中抗CGRP拮抗剂抗体G1的长期作用

[0671] 该实验的目的是确定抗CGRP抗体在给药之后7天是否仍可阻断电刺激的MMA扩张。

大鼠的制备与上述急性实验(实施例6)相同,以下除外。在产生闭合颅骨窗口准备和刺激之前7天,向大鼠i.v.注射(10mg/kg、3mg/kg或1mg/kg G1)。如在急性实验中那样,在给药之前不能建立对电刺激的基线扩张响应,因此将抗体组与在载剂(PBS,0.01%Tween 20)给药的对照组中的MMA扩张进行比较。使大鼠休息不少于45分钟后,以30分钟的间隔电刺激硬脑膜。刺激为2.5V、5V、10V、15V和20V,全部为10Hz、0.5ms脉冲持续30秒。

[0672] 如图10中所示,10mg/kg和3mg/kg的G1显著阻断了由10至20 伏的电刺激诱发的MMA扩张。该数据表明G1可在给药之后多至7天阻断电刺激的MMA扩张。

[0673] 实施例8:吗啡戒断热潮红模型(Morphine withdrawal hot flush model)

[0674] 吗啡戒断大鼠模型是用于绝经后热潮红机制的已建立的啮齿动物模型(Sipe等,Brain Res.1028(2):191-202(2004);Merchenthaler等,Maturitas 30:307-316(1998);Katovich等,Brain Res.494:85-94(1989);Simpkins等, Life Sciences 32:1957-1966(1983))。基本上,通过在皮肤下植入吗啡丸粒使大鼠对吗啡成瘾。成瘾后,向动物注射纳洛酮(阿片样拮抗剂),其立即使它们进入戒断。这种戒断伴随着皮肤温度升高、核心体温降低、心率提高以及血清黄体生成素提高。这些在量级和时间上都与人热潮红中发生的那些类似(Simpkins等,Life Sciences 32:1957-1966(1983))。此外,如果在诱导戒断前用雌二醇处理大鼠,则热潮红症状减轻(Merchenthaler等, Maturitas 30:307-316(1998))。这就是为什么吗啡戒断模型被认为是模拟临床热潮红的原因。

[0675] 从Charles River Laboratories订购去卵巢大鼠(ovariectomized rat)。在卵巢切除后不少于7天,通过皮下植入吗啡丸粒(75mg吗啡碱)产生吗啡依赖性。两天后,再植入两个丸粒。次日用10mg/kg 4901[**]或载剂 (PBS,0.01%tween)对大鼠进行静脉内注射。第二次丸粒植入之后两天,将大鼠用氯胺酮(90mg/kg)麻醉并轻轻束缚。将表面温度热电偶黏贴至尾部基部,并使用直肠热电偶来测量体核温度。使用图表软件 (ADInstruments)记录数据。记录15分钟稳定的基线温度之后,皮下注射纳洛酮(1mg/kg)。在接下来的60分钟内连续记录温度。结果示出在图11A和11B中。

[0676] 实施例9:非临床毒理学和药动学

[0677] 在Sprague-Dawley(SD)大鼠和食蟹猴(cynomolgus monkey)的1 个月IV重复剂量毒性研究中,抗CGRP拮抗剂抗体G耐受良好,并且在这些研究中均未测定到靶器官毒性。对于大鼠和猴研究二者建立了100 mg/kg/周的无不良事件水平(no adverse event level,NOAEL)。该剂量水平对应于大鼠和猴中分别最大浓度(Cmax)为2,570和3,440μg/mL并且曲线下面积(AUC(0-168h))为194,000μg·h/mL和299,000μg·h/mL(第 22天)的全身暴露。

[0678] 在3个月的IV/SC大鼠研究中,没有鉴定出靶器官毒性,且G1在直到300mg/kg的最高受试剂量下均良好耐受。在3个月的猴研究中,在≥100 mg/kg时观察到由于免疫复合物沉积导致的睫状动脉的血管周围炎症。这一发现归因于猴对人源化抗体的免疫原性响应,并且不被认为是临床相关的。在该猴研究中,300mg/kg的最高受试剂量是2,000mg或以mg/kg为基础的29mg/kg的最高预期临床剂量的至少10倍高(假定平均对象体重为70kg)。

[0679] 实施例10:临床药动学

[0680] 在检查10至2,000mg的剂量的四个随机化、安慰剂对照、双盲研究中检查单次IV暴露后抗体G1的PK。在1小时IV输注结束后不久达到最大血浆浓度(Cmax)。达到Cmax的中位时间(Tmax)为1.0至3.0小时,随后是多阶段下降。随着G1剂量的逐渐提高,Cmax和总暴露近似

线性提高。终末半衰期($t^1/_2$)为36.4至48.3天。肝中没有G1代谢的证据，代谢的主要模式通过蛋白质酶体降解。

[0681] 一个研究定义了以两周间隔给予两次的30mg和300mg剂量的药动学。最大浓度和浓度-时间曲线下面积随着剂量的提高而提高。第二次剂量之后的表观终末半衰期($t^1/_2$)为41.2天(30mg)和50.0天(300mg) (算术平均值)。以15天间隔施用两个IV剂量之后G1的血浆积累率为 1.5(30mg)和1.4(300mg)。

[0682] 实施例11:临床安全性和药动学

[0683] 在六项研究中，向118位健康男性和女性施用抗体G1，而57位男性和女性对象接受安慰剂。该研究包括0.2mg至2,000mg的单次IV剂量，每14天一次给予的多至300mg的两次IV剂量，以及225和900mg的 SC施用。六项研究包括：健康男性中的两项IV单次剂量递增PK和药效学(pharmacodynamics，PD)研究(研究B0141001和B0141002)；两组安慰剂对照的交叉研究，以检查健康志愿者中IV施用抗体G1对辣椒素潮红响应(capsaicin flare response)的急性效应(B0141006)；健康男性和女性志愿者中抗体G1的平行组重复剂量研究(B0141007)；单次剂量研究，其评价向健康女性志愿者施用的多至2,000mg的剂量的安全性和耐受性(B0141008)，以及比较IV施用和SC施用之间的相对安全性和生物利用度的研究(G1-SC-IV)。

[0684] 这六项研究总结在下表8中。在这五项IV研究(B0141001、B0141002、B0141006、B0141007和B0141008)中，三项具有几乎完全相同的设计和评估。研究B014100测试作为单次一小时IV输注给予的0.2mg、1mg、和3mg的剂量。研究具有平行设计。参与者在输注后被限制在诊所中持续七天，在这些天的每天进行多项评估。出院后，在出院后一周(第14 天)以及随后在输注后一、二和三个月重新评估患者。研究B0141002测试作为单次施用的10mg至1000mg的剂量。最后，研究B0141008测试了300mg、1000mg、1500mg或2000mg的剂量。研究B0141006与其他研究不同，因为其还旨在在抗体G1的IV输注之后通过测量辣椒素潮红抑制多至1周来整合药效学读出。

[0685] 对于IV研究，仅在首次给药期报道了不良事件(adverse event，AE) 概况。研究B0141007使用平行设计测试以两周间隔给予30或300mg IV 的多剂量抗体G1。通过包含处理分配的交互式基于Web的系统为每位合格对象分配随机化顺序。随机化模式由主要统计人员(lead statistician)开发。所有研究的参与者是一般健康的男性和女性(18至65岁)；所有参与者签署了知情同意书。所有研究均由调查审查委员会(investigation review board，IRB)批准。AE定义为临床研究参与者的任何不良医疗事件，其与所研究药物存在或不存在因果关系。所研究药物或安慰剂施用之后观察到的AE被称为“治疗突发(treatment-emergent)”AE(TEAE)，而不管与所研究药物的潜在因果关系。经历TEAE的所有对象以适当的时间间隔进行随访，直到事件已经解决或者事件已经稳定和/或达到新的基线。所有TEAE被评为轻度、中度或严重。严重AE(serious AE，SAE)将推理(a priori)定义为，在任何剂量下导致死亡的任何不良医疗事件是威胁生命的(即，事件发生时该对象处于立即死亡的风险中)，需要患者住院治疗或延长现有住院治疗，导致持久的或重大的残基/无行为能力(例如，对象完成正常生活功能的能力的实质性破坏)，导致先天性异常/出生缺陷，或任何其他医学上重要的事件。当存在以下情况之一时，考虑治疗相关的AE(treatment-related AR，TRAE)：1)可确定AE发作与研究产品施用之间似乎可信的时间关系；2)AE可不易通过患者的

临床状态、并发疾病或伴随治疗来解释;以及3)在停止所研究产品或剂量降低时AE减轻。

[0686] 在筛选时、给药前、输注结束后立即、患者限制在诊所期间的多个时间以及所有诊所随访时,测量血压、脉搏率和口腔温度。实验室检测包括血清化学、血液学和尿分析。在多个研究时间进行血液学、化学、凝血和尿安全实验室测试。在筛选时、第1天给药前、第一天期间输注结束后立即和五个其他时间以及在临床随访时记录ECG。使用Fridericia的(QTcF) 心率校正公式推导QTcF值。通过组群、治疗和给药后时间评估ECG参数QT间期、心率、QTcF间期、PR间期和QRS间期的绝对值和与基线相比的变化。除上述安全性评估外,方案B014008还包括在基线和给药之后的三个时间点(第28天、第84天和第168天)的完整眼科评估。

[0687] 使用描述性表格和汇总统计来总结临床数据和生命体征。实验室和其他安全性数据总结为任何变化(超出参考范围的值)以及推理定义的任何临床相关变化的函数。总结性表格按照剂量进行分层,并且将多个研究的数据汇集。另外,所有抗体G1暴露的合并数据的比较与安慰剂进行对比。安慰剂还与100mg和更高(100mg、300mg、1000mg、1500mg和2000mg)的抗体G1剂量以及1000mg和更高(1000mg、1500mg和2000mg) 的抗体G1剂量进行对比。

[0688] 在IV/SC研究(G1-SC-IV)中,36名对象随机接受作为皮下(SC) 推注或1-小时IV输注递送的抗体G1(225或900mg)或安慰剂的单次施用。给药之后对象限制在临床研究单位中7天,并且定期返回诊所用于额外门诊随访直至研究第90天。在限制对象的时候在第1天(给药前、第 1小时、第6小时、第12小时)、第3天和第7天广泛地进行ECG,并且在研究结束时进行一次(第90天)。在给药前、第1天、第3天、第7天和第90天收集生命体征,包括温度、血压和心率。

[0689] 表8

[0690]

研究	所研究人群	用抗体G1的处理
B0141001	健康成年男性（n = 24）	在8个的组群（6/组群，主动治疗；安慰剂组群中2个）中单次静脉内（IV）输注0.2、1或3 mg
B0141002	健康成年男性（n = 40）	在8个的组群（6/组群，主动治疗；安慰剂组群中10个）中单次IV输注10、30、100、300或1000 mg
B0141006	健康成年男性（n = 12）	两个组群修改的交叉安慰剂或6个的组群中300 mg IV输注。在第一阶段，所有参与者接受安慰剂。对于第二阶段（本文中包含），12位参与者接受安慰剂，并且11位接受300 mg。
B0141007	健康成年男性和女性（n = 21）	在10个或11个的组群中以两周间隔两次IV输注30或300 mg（6/组群，主动治疗；安慰剂组群中9个）
B0141008	健康成年女性（n = 31）	单次IV输注300、1000、1500或2000 mg（2000 mg组群中5个；6/组群保持治疗组；安慰剂组群中8个）
G1-SC-IV	36位对象（n = 36）	单次皮下（SC）推注或单次IV输注225或900 mg

[0691] 在五项IV研究(0.2至2,000mg)中评价的宽范围剂量中,IV抗体 G1可接受地耐受。表9总结了IV研究的剂量的总体不良事件(AE)率。基于这些耐受性结果,未出现公开的安全问题。在IV研究的所有试验中,接受安慰剂的参与者报告了平均1.3个治疗突发不良事件(TEAE)。无论调查人员对所研究药物的关系的意见如何,这些是所有报告的事件。在所有IV-G1剂量中,该比例为1.4TEAE/对象。接受100mg或更高的G1剂量的对象具有平均1.5个TEAE;接受1,000mg或更高剂量的那些具有平均1.6个TEAE。

[0692] 表9

[0693]

	评价AE的对象	AE数 - n (N)	具有AE的对象 - n (N)	具有严重AE的对象 - n (N)	具有严重AE的对象 - n (N)	由于AE而停止的对象 - n (N)	剂量降低或暂时停止 - n (N)
安慰剂	45	57 (11)	23 (8)	0	0	2 (1)	0
0.2mg	6	5 (0)	2 (0)	0	0	0	0
1mg	6	1 (1)	3 (0)	0	0	0	0
3mg	6	10 (2)	4 (1)	0	0	0	0
10mg	6	5 (1)	4 (1)	0	0	0	0
30mg	12	21 (11)	8 (5)	0	0	0	0
100mg	6	5 (1)	4 (1)	0	0	0	0
300mg	29	47 (10)	20 (7)	1 (1)	1 (1)	0	0
1000mg	12	17 (4)	8 (4)	0	0	0	0
1500mg	6	8 (0)	3 (0)	0	1 (0)	0	0
2000mg	5	12 (2)	4 (1)	0	0	0	1 (1)

[0694] AE＝不良事件;n＝与治疗相关或不相关的任何事件;(N)＝调查人员认为相关的治疗。注:对于方案B0141006(安慰剂和300mg),由于其交叉性质,仅包括第一主动治疗期的数据

[0695] 在IV研究中,与接收安慰剂的那些中的17.7%相比,21.2%的接受IV G1的对象中报告了治疗相关不良事件(TRAE,或根据主要调查人员可能与治疗相关的AE)。在100mg或更高剂量G1下,在22.4%的参与者中出现TRAE。在1,000mg或更高剂量下,在21.7%的参与者中出现TRAE。抗体G1看来不与任何临床相关生命体征变化模式相关(收缩压和舒张压[BP]、体温和心率[HR])、心电图(ECG)异常(包括QTcB和QTcF)、输注部位反应或临床实验室结果。对于肝功能测试(天冬氨酸转氨酶 [AST]、丙氨酸转氨酶[ALT]、总胆红素和碱性磷酸酶)的影响有限,在一位接受安慰剂的对象(研究B0141001)中总胆红素升高1级,且在一位接受安慰剂的对象(研究B0141002)中ALT升高1级。在接受任何所研究剂量的G1的对象中未观察到临床显著的肝功能异常。在评估肾功能的血液学测试、电解质或尿测试中,没有证据表明G1与安慰剂之间的差异。

[0696] 在IV/SC研究(G1-SC-IV)中,SC与IV递送途径之间的安全性和耐受性相当。平均心率和血压(舒张压和收缩压)不受抗体G1治疗的影响,用SC抗体G1治疗之后任何心血管参数都没有任何有意义的变化。SC研究期间观察到的TRAE总结如下在表10中示出。

[0697] 表10

[0698]

	900mg (N＝6)	225mg (N＝6)	Placebo (N＝6)
GI疾病	2(33.3%)	0	1(16.7%)
CNS	0	1(16.7%)	0

感染和侵染	0	0	0
肌肉骨骼和结缔组织	0	0	0
呼吸	0	0	0
生殖和乳房疾病	0	0	0
损伤	0	0	0
妊娠	0	0	0
肾	1(16.7%)	0	0
血管	0	0	0

[0699] 在单次剂量研究(B0141001、B0141002、B0141006和B0141008)中,计算30mg至2,000mg剂量的药动学(pharmacokinetics,PK)参数。组平均终末半衰期($t_{1/2}$)为约40至48天。Cmax和总暴露(通过AUC_{inf}评估)随着剂量的提高而提高。AUC_{inf}的提高看来大约为30至1,000mg 的剂量比例,并且看来大于1000至2,000mg的剂量比例。分布体积低,为6-10L。

[0700] 在两次剂量研究(B0141007)中,第二次剂量之后的表观终末半衰期为41至50天。第二次剂量之后血浆浓度累积,累积率为约1.5。此外,在IV/SC研究(G1-SC-IV)中,药动学评估表明G1在SC递送时具有与 IV类似的终末半衰期。

[0701] 实施例12:非临床安全性

[0702] 在食蟹猴中进行了两项评估抗体G1安全性的研究。在第一项研究中,评价单次剂量抗体G1的安全性。在第二项研究中,评价了重复剂量抗体 G1的安全性。下面将进一步详细描述每项研究及其结果。对于单次剂量和重复剂量研究二者,将抗体G1配制成在pH～5.5的20mM组氨酸、84 mg/mL海藻糖二水合物、0.2mg/mL聚山梨酯80、0.05mg/mL EDTA二钠二水合物和0.1mg/mL L-甲硫氨酸中的51.4mg/mL溶液。载剂相同地配制但没有抗体G1。此外,在这两项研究中,定期采集血液样品并使用经验证的ELISA方法分析抗体G1血浆浓度。

[0703] 首先使用GraphPad Prism(版本6.0)和Excel 2010(Microsoft)将数据汇总在总结表格和图中。对于单次暴露研究,使用ANOVA分析遥测数据。使用SAS Release 8.2进行分析。为了将QT间期相对于一系列R-R间期归一化,通过将每个RR间期与其相关的QT间期相关联来产生每只动物的个体动物校正因子(Individual Animal Correction Factor,IACF)。对于数据集确定了这种QT/RR间期关系的线性回归。这种线性回归的斜率用作所有处理中相关动物的IACF。该IACF被用于使用以下方程来计算校正的QT间期(QTc):

[0704] QT-I(c)＝针对心率进行校正的QT间期＝QT-I-[(RR-300)*(IACF)]。

[0705] 对于多剂量研究,还使用单因素ANOVA来分析数据。如果ANOVA 是显著性的($P \leq 0.05$),则Dunnett事后检验用于组间比较。对于每种性别,在5%双尾概率水平将处理组与对照(载剂)组进行比较。

[0706] 单次剂量遥测研究

[0707] 将八只成年雄性食蟹猴(Charles River Primates)经手术配备遥测仪(telemeter),并允许恢复至少两周。植入物(DSI TL11M2-D70-PCT)和接收器(RMC-1)由Data Sciences International制造。

[0708] 在给药前使动物至少过夜适应遥测数据采集笼。在适应期间,进行血液动力学参数的研究前记录以验证传感器和设备正确运行。在遥测数据采集期间,将动物单独安置在装有遥测接收器的笼中。在非收集日,将动物被安置在没有遥测接收器的笼中。将动物维持

在12小时光照、12小时黑暗的日循环，随意饮水和进食经认证的灵长类饮食。

[0709] 对于研究的第一阶段，向动物(8只雄性)仅施用载剂，并且在给药前约1小时开始至给药之后22小时收集遥测数据。载剂施用之后六天，相同动物接受单次IV施用的抗体G1(100mg/kg，为食蟹猴中的药理学 EC50的约10倍高的剂量)。再次从所有动物连续记录遥测心电图和血流动力学数据。另外，在接受其单次剂量的抗体G1之后，在第3、7、10 和14天监测这些动物约24小时。遥测ECG和血压信号通过植入的无线电遥测装置传送至安装在每个笼中的接收器。采集的信号通过数据交换矩阵(DSI模型DEM)并传送至基于PC的数据采集系统(DSI软件Ponemah P3版本3.4)；数据分析软件是Emka Technologies version 2.4.0.20(Emka Technologies)。模拟/数字采样率对于遥测ECG数据为1,000Hz，且对于血压数据为500Hz。数据作为一分钟的平均值记录。

[0710] 在整个第一天给药之后，以及随后的日子(在第3、7、10和14天以与第1天相同的时间间隔遥测动物)，用抗体G1处理之前和之后的组平均收缩压(systolic blood pressure，SBP)类似。在给药之后1至4小时当抗体G1血液浓度为最高水平(4小时时的平均浓度为3,500μg/mL)时，平均SBP为111mmHg，相比之下载剂施用之后相同时间间隔为113mmHg。此外，在抗体G1施用之后，第3天和第7天的SBP为110mmHg，第10 天为109mmHg，且第14天为110mmHg。其他时间间隔记录了类似的 SBP数据。由于这是交叉设计的研究，经处理动物作为其自身的对照。当与载剂处理相比抗体G1施用之后分析的数据为血压差时，在第7、10和14天的随后时间间隔的SBP有轻微的统计学显著性降低。

[0711] 在用抗体G1处理后，观察到舒张压(diastolic blood pressure，DBP) 比载剂施用之后获得的平均值低约3mmHg。从5-22小时开始，载剂组和抗体G1组的平均值类似。在其他天出现了同样的趋势，在第7-10天在 7-22小时间隔中，当第一次测量间隔中发生DBP的轻微下降(2.62-3.5 mmHg)时，零星发现类似量级的小的变化。与对于DBP所见相似，在第一次评估(1-4小时)期间发现相对于载剂处理心率的轻微下降。在中间评估期间差异无法检测到，并且在所有天的18-22小时之间再次出现。

[0712] 此外，关于ECG研究结果，相对于载剂处理，在任何时间点QTc间期均无统计学显著性变化。尽管在14天时间内与载剂相比在RR、PR、 RS和QT中具有统计学显著性变化，但它们的绝对值都很小。

[0713] 重复剂量安全性研究

[0714] 重复剂量安全性研究包括48只成年性别匹配的(每组每种性别6只) 未接受过抗体G1的食蟹猴(Charles River Primates)。动物每周一次以10 mg/kg、100mg/kg或300mg/kg的剂量接受静脉内注射的载剂或抗体G1 持续14周。在每组中，允许每种性别的两只动物在给药结束后允许恢复额外4个月。

[0715] ECG和血压测量在研究前阶段记录一次，达到稳态后(第85天给药前和给药之后4小时)记录两次，并且在给药结束之后约1周(恢复阶段第103)记录一次。将动物用氯胺酮麻醉，并使用八导联记录ECG。使用 Life Science Suite Ponemah Physiology Platform软件系统通过DSI使用导联I、II、aVF、CG4RL和CV4LL作为标准利用获取的数据进行ECG(包括心率)的测量。使用Bazett公式计算QT间期(QTc)的心率校正。

[0716] 在第一次给药前、给药12周后(13个剂量)和给药结束后约1周记录血压。相对于载剂处理的动物，在任何经处理动物组中未记录到SBP 或DBP的显著性变化。在测量的剂量组

和时间点,组平均心率相对一致,没有测量到统计学差异。在给药的第一周期间以及在血压和ECG评估时测量抗体G1的血浆浓度,表明随着每周重复给药而积累。

[0717] 此外,对于ECG研究结果,在所有剂量和时间点,QTc间期没有显著性差异。另外,对于在研究过程中评估的任何ECG参数,未观察到显著性或相关的ECG变化。

[0718] 总之,在这两项研究中抗体G1的耐受性很好,任何血流动力学参数都没有出现临床上显著性的变化,也没有任何ECG参数中记录的相关变化。在食蟹猴中,心血管和血液动力学参数似乎没有受到用抗体G1长期抑制CGRP的影响。

[0719] 实施例13:2期研究

[0720] 2期、多中心、随机化、双盲、安慰剂对照、平行组研究相对于安慰剂比较了皮下施用抗体G1(TEV-48125)的4个剂量方案对于治疗创伤后头痛的效力和安全性。

[0721] 主要终点:

[0722] 主要终点是所研究药物的最后一个(第3)剂量之后的28天时期期间任何严重程度的头痛小时数相对于基线(28天磨合期(run-in period)) 的平均变化。

[0723] 次要终点:

[0724] -用所研究药物治疗3个月期间实现任何严重程度的每月头痛日数至少50%降低的患者的比例

[0725] -所研究药物的第一剂量之后28天的时期期间任何严重程度的头痛小时数相对于基线(28天磨合期)的平均变化

[0726] -所研究药物的最后一个(第3)剂量之后28天的时期期间任何急性头痛药物的使用相对于基线(28天磨合期)的平均变化

[0727] -所研究药物的最后一个(第3)剂量施用之后28天时通过6项头痛影响测试(HIT-6)测量的失能评分相对于基线(第0天)的平均变化

[0728] 样本大小

[0729] 150位患者(每组30位)。

[0730] 研究组

[0731] -组1.安慰剂

[0732] -组2.TEV-48125 SC每月225mg

[0733] -组3.TEV-48125 SC每月675mg

[0734] -组4.TEV-48125 IV 675mg一个剂量

[0735] -组5.TEV-48125 IV 1000mg一个剂量

[0736] 纳入标准

[0737] 根据ICHD 3,参与者具有慢性创伤后头痛病史。

[0738] 排除标准:

[0739] -目前登记在或者在最近30天内终止涉及任何所研究药物或装置的临床试验。

[0740] -目前使用或任何先前暴露于任何降钙素基因相关肽(CGRP)抗体、任何抗CGRP受体的抗体或抗神经生长因子(NGF)的抗体。

[0741] -无法进行超过4次足够的预防性药物试验。

[0742] -已知对多种药物、单克隆抗体或其他治疗性蛋白质过敏。

[0743] -存在表明会妨碍研究参与之医学问题的其他医学疾病或有其病史。

[0744] -根据调查人员的意见，显著活跃或不稳定的精神疾病的证据。

[0745] -妊娠或哺乳的妇女

[0746] 表11：研究程序和评估

[0747]

研究时期	治疗前时期（包括筛选随访和磨合期）	双盲治疗时期				随访时期
随访编号	V1[a)]	V2[b)]	V3	V4	V5[c)]	V6[d)]
月编号	第-1 月	第 0 月	第 1 月	第 2 月	第 3 月	第 9.5 月
程序和评估[e)]	筛选 第-28 至-1 天	基线 剂量 1 第 0 天 （+3 天）	剂量 2 第 28 天（±3 天）	剂量 3 第 56 天（±3 天）	EOT 或早期退出 第 84 天（±3 天）	最后随访 第 281 天（±15 天）
知情同意书	X					
病史和精神病史	X					
先前的用药史	X					
纳入标准和排除标准	X	X				
体检，包括体重和身高[f)]	X	X			X	
12-导联 ECG[g)]	X	X	X	X	X	
生命体征测量[h)]	X	X	X	X	X	
不良事件[i)]	X	X	X	X	X	X
伴随用药询问	X	X	X	X	X	X
临床实验室测试[j)]	X	X	X	X	X	
血清/尿 β-HCG 测试[k)]	X	X			X	
FSH[l)]	X					
电子头痛日志[m)]	X	X	X	X	X	
用于血浆药物浓度的血液样品		X	X	X	X	X
用于血清 ADA 浓度的血液样品		X	X		X	X
用于药物基因组学分析的血液样品[n)]		X				

[0748] 表11：研究程序和评估(续)

[0749]

研究时期	治疗前时期（包括筛选随访和磨合期）	双盲治疗时期				随访时期
随访编号	V1[a)]	V2[b)]	V3	V4	V5[c)]	V6[d)]
月编号	第-1 月	第 0 月	第 1 月	第 2 月	第 3 月	第 9.5 月
程序和评估[e)]	筛选 第-28 至-1 天	基线 剂量 1 第 0 天 （+3 天）	剂量 2 第 28 天(±3 天）	剂量 3 第 56 天(±3 天）	EOT 或早期退出 第 84 天（±3 天）	最后随访 第 281 天（±15 天）
血液收集用于进一步生物标志物分析		X		X	X	
尿收集用于进一步生物标志物分析		X		X	X	
DISABILITY (TBD)		X			X	
COGNITION (TBD)		X			X	
QOL (TBD)		X			X	
PGIC 调查表			X	X	X	
所研究药物的施用		X	X	X		
注射部位评估[o)]		X	X	X		

[0750] 实施例14:2期研究

[0751] 2期、多中心、随机化、双盲、安慰剂对照、平行组研究相对于安慰剂比较了TEV 48125的两种给药方案(一种皮下和一种静脉内剂量方案) 对于治疗持续性创伤后头痛(persistent post-traumatic headache,PPTH) 的效力和安全性。

[0752] 所研究人群

[0753] 所研究人群由男性和女性患者构成,年龄为18岁至70岁(包括端值),具有持续性创伤后头痛病史(根据International Classification of Headache Disorders,第三版[ICHD-3]标准(IHS 2013)定义。

[0754] 这将包括因头部轻度创伤性损伤引起的持续性头痛患者以及因颈部扭伤(whiplash)引起的持续性头痛患者。

[0755] 头部的创伤性损伤定义为外力在头部的作用导致的结构性或功能性损伤。这些包括用物体撞击头部或头部撞击物体、头部被外来物穿透、由爆炸或爆破产生的力、以及尚未确定的其他力。

[0756] 创伤后遗忘症的持续时间定义为头部损伤与恢复对当前事件以及过去24小时内发生的事件的记忆之间的时间。

[0757] PPTH是轻度头部损伤造成的头痛,格拉斯哥昏迷量表评分(GCS) 为13至15,意识丧失少于30分钟,且创伤后遗忘症的持续时间少于24 小时,所述创伤后遗忘的持续时间定义为头部损伤与恢复对当前事件的记忆之间的时间。此外,两种或更多种其他症状表明轻度创伤性脑损伤:恶心、呕吐、视觉障碍、头晕和/或眩晕、记忆力和/或注意力受损。

[0758] 颈部扭伤造成的持久性头痛是在颈部扭伤之后7天内发生并且持续超过三个月的

头痛。颈部扭伤在当时与颈部疼痛和/或头痛相关。颈部扭伤定义为伴有颈部弯曲/伸展的头部突然且未适当限制的加速/减速运动。在高或低冲击力时可发生颈部扭伤。

[0759] 研究终点

[0760] 主要终点:主要终点是施用所研究药物之后4周时期期间至少中度严重程度的每月平均头痛日数相对于基线(28天的磨合期)的平均变化。

[0761] 次要终点:次要终点是:

[0762] ●用所研究药物的12周治疗时期期间实现任何严重程度的每月头痛日数至少50%降低的患者比例

[0763] ●所研究药物的第一剂量之后5至8周时期期间任何严重程度的头痛日数相对于基线(28天磨合期)的平均变化

[0764] ●所研究药物的第一剂量之后12周时期期间任何严重程度的头痛日数相对于基线(28天磨合期)的平均变化

[0765] ●施用第一次所研究药物之后12周时通过6项头痛影响测试(HIT-6) 测量的失能评分相对于基线(第0天)的平均变化。

[0766] 探索性终点:探索性终点如下:

[0767] ●所研究药物的第一剂量之后12周时期期间至少中度严重程度的头痛日数相对于基线(28天磨合期)的平均变化

[0768] ●在所研究药物的第一剂量之后12周时期期间实现任何严重程度的每月头痛日数至少75%降低或完全(100%)降低的患者的比例

[0769] ●在所研究药物的第一剂量之后4周时期期间实现任何严重程度的头痛日数相对于基线时期至少50%降低、至少75%降低并且在所研究药物的第一剂量之后12周时期保持该响应水平的患者的比例

[0770] ●所研究药物的第一剂量之后12周时期期间任何严重程度的每月平均头痛日数相对于基线(28天磨合期)的平均变化

[0771] ●所研究药物的第一剂量之后4周时期期间至少中度严重程度的每月平均头痛日数相对于基线(28天磨合期)的平均变化

[0772] ●在所研究药物的第一剂量之后8周时期期间实现至少中度严重程度的每月平均头痛日数相对于基线时期至少50%降低、至少75%降低或完全(100%)降低的患者的比例

[0773] ●仅对于颈部扭伤患者,所研究药物的第一剂量之后12周时期期间任何严重程度的每月平均颈部疼痛天数相对于基线(28天磨合期) 的平均变化

[0774] ●施用第一所研究药物之后12周时期期间任何急性头痛药物(曲普坦类和麦角化合物)的使用相对于基线(28天磨合期)的平均变化

[0775] ●所研究药物的第一剂量之后12周时期期间阿片类的使用相对于基线(28天磨合期)的平均变化

[0776] ●施用第一所研究药物之后四周时通过6项头痛影响测试(HIT-6)测量的失能评分相对于基线(第0天)的平均变化

[0777] ●所研究药物施用的第一剂量之后四周时通过12项简式健康调查 (SF-12)身体和精神健康测量的健康状况相对于基线(第0天) 的平均变化

[0778] ●施用所研究药物的第一剂量之后4、8和12周时通过患者变化总体印象(PGIC)量

表测量的患者满意度评估相对于基线(第0天) 的平均变化

[0779] ●所研究药物的第一剂量之后12周评价时期结束时通过SCAT-3“运动震荡评估工具-第3”测量的相对于基线(第0天)的平均变化

[0780] 一般设计和方法：

[0781] 研究程序和评估见表12。

[0782] 多中心、随机化、概念验证、双盲、安慰剂对照、平行组研究相对于安慰剂比较了TEV 48125的两种剂量方案(1种皮下和1种静脉内)施用对于诊断持续性创伤后头痛(PTH)的效力和安全性。研究将由筛选随访、28天磨合期和持续约12周的双盲治疗期组成。

[0783] 患者将在提供书面知情同意书之后完成筛选随访(随访1)，并且符合条件的患者将进入持续大约四周(28天)的磨合期，在此期间他们将每日将基线持续性创伤后头痛(PPTH)发作信息输入电子头痛日志装置。患者在完成磨合期之后返回研究中心(随访2)。在筛选随访时和接下来的28天磨合期中符合资格要求的患者随机分至三个治疗组之一中：

[0784] 一个治疗组每季度在一小时内接受900mg IV输注的第一剂量 TEV-48125：

[0785] 一个治疗组接受675mg SC的第一剂量TEV-48125，随后在接下来2 个月接受225mg SC;以及

[0786] 一个治疗组接受三个每月剂量的安慰剂。

[0787] 随机化将使用电子交互式响应技术(interactive response technology, IRT)进行。

[0788] 盲法治疗在第一个月IV和SC施用并且在第二和第三个月SC施用总计三个剂量。第一治疗施用在随访2时进行，并且额外剂量在随访3和4 时施用。患者将大约每四周返回研究中心进行SC施用的盲法治疗用于安全性和有效性评估，以及血液和尿取样用于药动学、免疫原性、生物标志物和药物基因组学(除非当地法规禁止)分析。最终研究评估将在该研究的最后一次随访(随访5)(在施用所研究药物之后的约12周)时进行。

[0789] 盲法和随机化方法：

[0790] 发起人(sponsor)、调查人员、研究职员(除了参与生物分析学分析的职员)和患者对治疗分配不知情。将计算机生成的主随机化清单提供给药物包装设施。包装供应商将根据药品生产质量管理规范(Good Manufacturing Practice)程序将活性剂和安慰剂包装到单一随访药盒中。药盒将在外观上完全相同，并且含有一个具有活性药物或安慰剂的小瓶和预填充注射器(活性剂或安慰剂)。为即将进行的研究随访供应足够的药盒将由IRT管理并在现场保存(冷藏在2℃至8℃)。

[0791] 在筛选期结束时，如果患者在运行期间具有至少6个或更多个至少中度严重程度的头痛日，以及至少85％的日志依从性，则将他们随机化。

[0792] 本研究是随机化研究，根据IHS分类，基于性别、头部创伤性损伤的严重程度(轻度、中度或严重)进行分层。通过IRT分配，每位患者将在他或她所属的层内以1:1:1的比例随机化以接受TEV-48125或安慰剂。IRT 将管理初始药物供应，维护现场充足的所研究药物供应，并集中研究随机化。

[0793] 所研究药物剂量、施用方式和施用率：

[0794] 将预填充小瓶(活性剂或安慰剂)将包含在唯一编号的药盒中，并在现场储存(冷藏在2℃至8℃)。用于IV施用的活性剂小瓶(10mL)将含有浓度为150mg/mL的TEV-48125，且

安慰剂小瓶(10mL)将含有与活性剂输注和注射相同的载剂和赋形剂。活性剂注射器将含有150mg/mL 的TEV-48125,且安慰剂注射器将含有与活性剂注射相同的载剂和赋形剂。预填充注射器(活性剂或安慰剂)和一个小瓶将被包含在唯一编号的药盒中。

[0795] 所研究药物将由合格的研究人员施用,并将检索适当编号的药盒并提取一定体积的小瓶内容物,并将其添加至500mL生理盐水溶液或SC注射剂中,并如下施用:

[0796] ●随机化为接受TEV-48125的患者在随访2时接受作为1小时IV输注的900mg TEV-48125和3次1.5mL安慰剂SC注射,并在随访 3和4时接受单次1.5mL安慰剂SC注射。

[0797] ●随机化为接受TEV 48125的患者将在随访2时接受作为三次活性剂 SC注射(225mg/1.5mL)的675mg和作为1小时IV输注的安慰剂,并在随访3和4时接受单次1.5mL 225mg活性剂SC注射。

[0798] 随机化为接受安慰剂的患者将在随访2时接受作为1小时IV输注的安慰剂和三次1.5mL安慰剂SC注射,并在随访3和4时接受单次1.5mL 安慰剂SC注射。

[0799] 研究产品:TEV-48125

[0800] 安慰剂:与活性剂相同的载剂和赋形剂

[0801] 患者参与的持续时间:患者参与将持续约16周(包括持续约4周的磨合期和12周的双盲治疗期)。预期患者完成研究的整个持续时间。

[0802] 纳入标准:如果患者符合以下所有标准,则可纳入研究:

[0803] a.参与者根据ICHD-3(β版)标准诊断为持续性创伤后头痛

[0804] b.已经发生对头部的创伤性损伤,其定义为外力在头部的作用导致的结构性或功能性损伤。这些包括用物体撞击头部或头部撞击物体、头部被外来物穿透、由爆炸或爆破产生的力、以及尚未确定的其他力。

[0805] c.在以下情况之一发生之后七天内报告发生了头痛:

[0806] 1.头部的轻度创伤性损伤

[0807] 2.头部损伤之后的意识恢复

[0808] 3.停止在头部损伤之后损害感觉或报告头痛之能力的药物

[0809] d.头部损伤之后头痛持续超过3个月。在颈部扭伤之后7天内发生颈部扭伤造成的持续性创伤后头痛(PPTH),并且持续时间超过3 个月。

[0810] 不超过30%的具有颈部扭伤的PPTH的患者将没有头部损伤证据。

[0811] e.患者在知情同意书上签名并签署日期。

[0812] f.男性或女性年龄为18至70岁(包括端值)

[0813] g.在磨合期期间患者具有至少6个或更多个至少中等严重程度的头痛日。

[0814] h.患者通过病史、医学体检、ECG、血清化学、血液学、尿分析和血清学确定为健康状况良好。

[0815] i.所有对象必须为无生育潜力,其定义为:

[0816] ○通过有记录的全子宫切除术、双侧卵巢切除术或双输卵管结扎而手术不育或者确认为绝经后(自最后一次月经至少一年并且卵泡刺激素[FSH]高于35U/L)的妇女;

[0817] ○通过有记录的输精管结扎手术而手术不育的男性;或者

[0818] ○如果具有生育潜力,对象必须符合以下任何标准:

[0819] ■在整个研究期期间以及所研究药物的最后剂量之后7.5个月内,对象与其伴侣

必须同时使用两种形式的高效避孕方法。

[0820] ■性禁欲仅在如果在限定时期定义为禁止异性性交时被视为高度有效的方法。需要根据临床研究的持续时间和对象的优选和通常的生活方式来评价性禁欲的可靠性。定期禁欲 (例如,日历法、排卵法、升温症状(symptothermal)法、排卵后法)、在研究期间宣布禁欲、以及戒断都不是可接受的避孕方法。

[0821] j.具有生育潜力女性对象在筛选时必须具有阴性血清β人绒毛膜促性腺激素(β-HCG)妊娠测试(通过在登记时的血清β-HCG妊娠测试证实)。

[0822] k.患者如果是男性是手术不育的,或者如果能够生育后代,其具有唯一同性伴侣或者目前正在使用经批准的避孕方法,并同意在研究期间继续使用该方法。可接受的避孕方法包括禁欲、女性伴侣使用类固醇避孕药(口服、植入或注射)与屏障方法的联合、女性伴侣使用宫内节育器、或如果女性伴侣手术不育。此外,男性患者在研究期间不能捐献精子。

[0823] l.患者必须愿意且能够遵守研究限制,并在研究期间保持在诊所持续期望的持续时间,并且愿意返回诊所进行本方案中规定的随访评价。

[0824] 排除标准:如果患者符合任何以下标准,将被排除参与本研究:

[0825] a.先前脑成像显示由于创伤性头部损伤造成的颅内出血、硬膜下或硬膜外血肿和蛛网膜下出血的病史。

[0826] b.开颅手术造成的PPTH

[0827] c.患者具有不是由于头部创伤造成的其他头痛疾病

[0828] d.具有任何类型的头痛(每月>6个头痛日)的先前病史(在头痛创伤之前)的患者

[0829] e.患者正使用含有阿片类(包括可待因)的药物和含巴比妥类的止痛药每月平均超过10天。

[0830] f.目前登记在或先前在最近30天参与涉及任何所研究药物或装置的临床试验,或5.5个半衰期(不论多长时间)

[0831] g.目前使用或任何先前暴露于任何降钙素基因相关肽(CGRP)抗体、任何抗CGRP受体的抗体或抗神经生长因子(NGF)的抗体。

[0832] h.已知对多种药物、单克隆抗体或其他治疗性蛋白质过敏。

[0833] i.存在表明会妨碍研究参与之医学问题的其他医学疾病或有其病史。

[0834] j.根据调查人员的意见,有重大活跃或不稳定的精神疾病的证据。

[0835] k.病人是妊娠或哺乳妇女。(任何在研究期间妊娠的妇女将退出研究。)

[0836] l.患者先前曾参与过Teva赞助的所研究药物的临床研究。

[0837] m.临床上重大的血液学、心脏、肾、内分泌、肺部、胃肠、泌尿生殖、神经、肝或眼部疾病,以及根据调查人员的判断患者具有任何临床上重大的不受控制的医学病症(经治疗或未治疗)。

[0838] n.由于以下任何原因,根据患者的医疗保健提供者或调查人员的意见,患者不能参与或成功完成研究:

[0839] ○心智上或法律上无行为能力或因任何原因而无法给出同意

[0840] ○由于行政或法律决定而受监禁、被监护或被收容至疗养院 (sanitarium)或社会机构

[0841] ○在紧急情况下不能联系到

[0842] ○有任何其他情况，其根据调查人员的意见使得患者不适合纳入研究

[0843] 量度和时间点：

[0844] 主要效力量度和时间点：本研究的主要效力终点将来自于使用电子头痛日志装置每天收集的持续性创伤后头痛日数据(即，疼痛日的出现、头痛的持续时间、头痛严重程度和急性头痛特异性药物使用)。

[0845] 符合条件的患者将接受关于使用电子头痛日志装置的培训，并将在筛选时获知合规要求。患者将每日完成关于前一日的问题的电子头痛日志条目，在筛选随访之后第二天开始直至EOT/早期撤出随访。电子头痛日志装置将允许在给定天之后多至24小时输入头痛信息。

[0846] 次要效力量度和时间点：次要效力终点将来自于使用电子头痛日志装置每天收集的头痛日数据(即，出现、头痛持续时间、头痛严重程度和急性头痛特异性药物使用)。此外，在第一剂量的所研究药物施用8周之后，患者感知的改善将通过头痛影响测试(HIT-6)评价。

[0847] 探索性效力量度和时间点：将评估以下探索性效力量度：

[0848] ●探索性效力终点来自于使用电子头痛日志装置每天收集的PTH日数据(即，头痛日的出现、持续时间、头痛严重程度和急性头痛特异性药物使用)。

[0849] ●头痛影响测试(HIT-6调查表)

[0850] ●SF-12身心健康

[0851] ●患者变化整体印象(PGIC)调查表

[0852] ●SCAT-3“运动震荡评估工具-第3

[0853] 安全性量度和时间点：

[0854] 使用以下量度评估安全性和耐受性：

[0855] ●关于不良事件的调查

[0856] ●关于伴随用药的调查

[0857] ●安全性实验室测试(血清化学、血液学、凝血和尿分析)

[0858] ●血清/尿β-HCG测试(仅限有生育潜力的妇女)

[0859] ●12导联ECG

[0860] ●生命体征测量(收缩压和舒张压、脉搏和口腔温度。在怀疑过敏的情况下应测量氧饱和度。在这些情况下也将测量呼吸率(但不作为标准生命体征)

[0861] ●体检，包括体重

[0862] ●超敏性评估

[0863] ●eC-SSRS

[0864] 药动学/生物标志物/免疫原性和生物标志物子研究量度和时间点：药动学量度和时间点：为了群体药动学建模方法和药动学/药效学关系评估，将从所有患者收集用于TEV-48125的药动学分析的血液样品。药效学参数将是效力响应。

[0865] 每份血液样品的实际日期和时间以及每份样品之前的给药日期和时间将记录在病例报告表中。TEV-48125血浆浓度将使用经验证的测定来测量。

[0866] 免疫原性量度和时间点：将收集用于免疫原性的血液样品。

[0867] 生物标志物量度和时间点：从所有患者收集生物标志物血液和尿样品，并且将在

随访2或其后的任何随访时从患者收集血液样品(除非当地法规禁止)。

[0868] 研究之前和期间允许和不允许的药物:

[0869] 除阿片类和巴比妥类之外,不论适应症和无流产剂限制,不超过2种药物(参考排除标准)

[0870] 统计学考虑:

[0871] 样本量基本原理:没有进行统计效力的预期计算。基于临床和实践考虑,选择75位患者(每个治疗组25位患者)的样本量。

[0872] 主要终点分析:将使用协方差分析(ANCOVA)方法分析施用主要效力终点:施用所研究药物之后四周时期期间至少中度严重程度的每月平均头痛日数相对于基线(28天的磨合期)的平均变化。该模型将包括治疗、性别、以及作为固定效应的头部创伤性损伤的严重程度(轻度、中度或严重)。对于每个TEV-48125组与安慰剂组之间的最小二乘均值差异,将构建百分之九十五的置信区间。

[0873] 次要和探索性终点的分析:对于连续的次要和探索性效力终点,将使用与主要效力终点相同的分析。对于定义为每月平均头痛日相对于基线 50%或更多降低的响应者的比例,将使用Cochran-Mantel-Haenszel检验。

[0874] 多重比较和多重性:将不进行多重比较。

[0875] 安全性分析:

[0876] 所有不良事件将使用Medical Dictionary for Regulatory Activities记录。在用于安全性分析的每个优选术语或系统器官种类类别中,每个患者将仅计数一次。将对所有不良事件(整体和按严重程度),由调查人员确定的与所研究药物相关的不良事件(定义为相关或缺失关系)(整体和按严重程度)、严重不良事件和造成退出研究的不良事件。将给出严重不良事件和导致退出的不良事件的患者列表。

[0877] 局部耐受性研究结果将被列出并描述性总结。

[0878] 描述性地总结实验室和生命体征测量数据的变化。所有值将与预先指定的边界进行比较以鉴定潜在的临床显著性变化或值,并且这样的值将被列出。

[0879] 伴随药物的使用将通过使用描述性统计学的治疗类别进行总结。伴随药物将包括在用所研究药物治疗患者时摄取的所有药物。

[0880] 安全性数据将整体和按照治疗组描述性总结。对于连续变量,将为每个时间点的实际值和相对于基线的变化提供描述性统计量(n、平均值、SD、中位数、最小值和最大值)。对于分类变量,将提供患者计数和百分比。还将提供严重不良事件、由于不良事件造成的患者退出以及基于预定标准的潜在临床显著性异常值(临床实验室或生命体征)的描述性总结。

[0881] 如果任何患者在研究期间死亡,将提供死亡列表,并且所有相关信息将在临床研究报告中的患者叙述中讨论。

[0882] 免疫原性分析:将提供免疫原性结果的总结,并将计算免疫原性的发生率。将评价免疫原性对药动学特征、药物效力和临床安全性的影响。该分析将单独地报告。

[0883] 生物标志物分析:生物标志物分析将包括逻辑回归、接受者操作特征曲线和概要统计。结果将单独地报告。将使用经验证测定进行测量。

[0884] 表12.研究程序和评估

[0885]

研究时期	治疗前时期（包括筛选随访和磨合期）	双盲治疗时期				随访时期
随访编号	V1[a)]	V2[b)]	V3	V4	V5[c)]	V6[d)]
月编号	第-1 月	第 0 月	第 1 月	第 2 月	第 3 月	第 9.5 月
程序和评估[e)]	筛选 第-28 至-1 天	基线 剂量 1 第 0 天（+3 天）	剂量 2 第 28 天(±3 天）	剂量 3 第 56 天(±3 天）	EOT 或早期退出 第 84 天（±3 天）	最后随访 第 281 天（±15 天）
知情同意书	X					
病史和精神病史	X					
先前的用药史	X					
纳入标准和排除标准	X	X				
体检，包括体重和身高[f)]	X	X			X	
12-导联 ECG[g)]	X	X	X	X	X	
生命体征测量[h)]	X	X	X	X	X	
不良事件[i)]	X	X	X	X	X	X
伴随药物查询	X	X	X	X	X	X
临床实验室测试[j)]	X	X	X	X	X	
血清/尿 β-HCG 测试[k)]	X	X			X	
FSH[l)]	X					
电子头痛日志[m)]	X	X	X	X	X	
用于血浆药物浓度的血液样品		X	X	X	X	X
用于血清 ADA 浓度的血液样品		X	X		X	X
用于药物基因组学分析的血液样品[n)]		X				

[0886] 表12:研究程序和评估(续)

[0887]

研究时期	治疗前时期(包括筛选随访和磨合期)	双盲治疗时期				随访时期
随访编号	V1[a)]	V2[b)]	V3	V4	V5[c)]	V6[d)]
月编号	第-1 月	第 0 月	第 1 月	第 2 月	第 3 月	第 9.5 月
程序和评估[e)]	筛选 第-28 至-1 天	基线 剂量 1 第 0 天 (+3 天)	剂量 2 第 28 天 (±3 天)	剂量 3 第 56 天 (±3 天)	EOT 或早期退出 第 84 天 (±3 天)	最后随访 第 281 天 (±15 天)
血液收集用于进一步生物标志物分析		X		X	X	
尿收集用于进一步生物标志物分析		X		X	X	
HIT 6 调查表		X			X	
		X			X	
SF 12 调查表		X			X	
PGIC 调查表			X	X	X	
所研究药物的施用		X	X	X		
注射部位评估[o)]		X	X	X		

[0888] 实施例15:创伤后头痛的临床相关大鼠模型中头痛相关行为的发生由CGRP介导

[0889] 材料和方法

[0890] 动物

[0891] 在所有研究中使用雄性Sprague-Dawley大鼠(Taconic,USA),到达时体重为250-300g。在室温下,将动物成对饲养在恒定的12小时光照/ 黑暗(在07:00h开始光照)循环下。食物和水可随意获得。在所有实验中,将动物随机分配至假手术组或轻度闭合性头部损伤(mCHI)组以及用于不同的处理,并且对于不同处理和组以盲法方式进行测试。所有实验均得到Beth Israel Deaconess Medical Centre和Harvard Medical School的机构动物护理和使用委员会(institutional Animal Care and Use Committee) 的批准并遵照其进行,并且遵照ARRIVE(动物研究:体内实验报告 (Animal Research:Reporting of In Vivo Experiments))指南。

[0892] 实验性轻度闭合性头部损伤

[0893] 如先前所述(Marmarou等,J of Neurosurgery 1994;80(2):291-300; Mychasiuk等,J Neurotrauma 2014;31(8):749-757),使用落重震荡装置 (wight-drop concussive device)诱导实验性mCHI。简单来说,将大鼠用 3%异氟烷麻醉并且胸部朝下放在落重震荡头部创伤装置正下方。该装置由垂直放置在老鼠头部上方的空心圆柱管(内径2.54cm)组成。为了诱导头部创伤,250g重物从80cm高度通过管落下,撞在头部中央。将泡沫海绵(厚度3.81cm,密度1.1g/cm^3)放置在动物下方,以在撞击运动时支撑头部,同时允许一些前-后运动(anterior-posterior motion),而没有任何旋运动。撞击后立即将动物返回笼中进行恢复。所有动物在损伤的2 分钟内恢复意识,并且在损伤后的早期数小时和数天中进行针对表明神经损伤之任何行为异常的神经病学评估。假手术动物进行麻醉,但不遭受落重。进行

PTH行为评估的所有动物均未显示出任何重大的神经病学缺陷。

[0894] 行为测试

[0895] 开放场地监测系统

[0896] 使用活动监测器SOF-811(Med Associates,Vermont,USA)来测量开放场地环境中的自主活动。系统由两个区域设置组成,并评价动物在水平平面(X-Y轴)和垂直平面(Z轴)平面上的运动。每个平面由相隔2.54 cm的16个束监测,并且位于每个场地每侧的每个束具有红外发射器和检测器,以监测在场地宽度上的运动。数据从3组检测器-发射器发送至中央计算机,其显示60秒间隔期间的一系列预选输出,包括总移动距离和垂直活动(竖起),并且总共分析超过20分钟。每个场地都用调光器开关 (dimmer switch)上的单个白色LED灯照明,以保持整个场地的均匀照明(80勒克斯(lux))。在连续的大鼠之间将场地用温和的洗涤剂清洁并干燥以去除气味线索。在基线(头部创伤之前24小时),并随后在mCHI 之后48小时、72小时、7天和14天进行测试。

[0897] 新物体识别

[0898] 设计该新物体识别测试以评价啮齿动物物识别记忆中的缺陷,作为脑损伤严重程度的量度。用于新物体识别测试的程序类似于先前描述的程序 (King等,Neuropharmacology 2004;47(2):195-204;Moriarty等, Behavioural brain research 2016;303:61-70),但进行了一些修改。设备由有机玻璃(Perspex)场地(45cm^2)构建。使用的“熟悉”物体是由“乐高DUPLO”积木构成的两个相同形状。第三个物体“新物体”由绿色带覆盖的塑料瓶组成。在测试天的前一天,在没有物体的情况下使动物适应场地30分钟。测试天包含三个阶段:适应(在没有物体的情况下暴露于场地5分钟)、暴露1(熟悉相同物体5分钟)和暴露2(暴露于一个熟悉物体的和一个新物体5分钟)。适应和暴露1间隔5分钟的试验间间隔,且暴露1和暴露2再间隔5分钟。对物体的探究定义为针对它的任何行为(即,嗅探、竖起、倚靠或攀爬)。记录三个测试阶段用于后续分析,并且以盲法方式手动评估物体探究。每个物体的辨别率作为探究任一物体花费的时间/探究这两个物体花费的时间计算。高于0.5(即,50%)的偏好指数指示新物体偏好,低于0.5-熟悉物体偏好,0.5本身-没有偏好。

[0899] 冯弗雷(Von Frey)测试

[0900] 所使用的方法先前用于研究头痛相关行为(Edelmayer等,Methods Mol Biol 2012;851:109-120;Oshinsky,Headache 2007;47(7):1026-1036;Yan 等,Mol Pain 2012;8:6,Zhao等,Pain 2014;155(7):1392-1400)。简单地说,将动物置于透明的平底丙烯酸容纳设备(20.4cm×8.5cm)中。该设备足够大,使得动物能够逃避刺激。测试前动物适应场地15分钟。为了确定动物在mCHI之后是否发生了颅骨膜机械超敏性,用不同冯弗雷细丝(0.6g-10g)(18011 Semmes-Weinstein Anesthesiometer Kit)刺激皮肤区域,包括眼上方中线区域和后部2cm。通过刺激后爪的中背部分测试后爪超敏性的发生。通过如下记录改编自Vos等(Journal of Neuroscience:the official joumal of the Society for Neuroscience 1994;14(5Pt 1):2708-2723)的四种行为响应,类似于先前的研究(Levy等,Brain,Behavior,and Immunity 2012;26(2):311-317)来评价触觉皮肤敏感性的变化:0)无响应:大鼠对刺激没有任何响应;1)察觉:大鼠将头转向刺激物体,并随后通常通过嗅探来探究;2)缩回:大鼠将头转离或将其迅速拉离刺激物体(通常随后刮擦或梳理刺激区域);3)逃离/攻击:大鼠在容纳设备中迅速转身以便逃离刺激或者攻击(咬或抓的动作)刺激物

体。从最低的重量开始，每个细丝以5秒的施加间隔施加三次，并且记录至少两次观察到的行为。对于统计学分析，记录的评分是基于记录的最厌恶的行为。引发连续三次缩回响应的力被认为是响应阈值。为了评价每个时间点每只大鼠除阈值变化以外的疼痛行为，通过组合每个测试的VF细丝的单独评分(0-3)来确定累积响应评分。所有测试都以盲法方式进行和评价。在基线以及在mCHI 之后的48小时、72小时以及7天和14天测试对冯弗雷刺激的响应。

[0901] 使用舒马普坦的条件化位置偏好

[0902] 所采用的方法改编自先前公开的报道(Griggs等，Neuroreport 2015;26(9):522-527)。使用由两个分开的室组成的两个CPP盒(Med Associates，Vermont，USA)来评估药物条件化阶段之前和之后的室偏好。选择舒马普坦用于初始表征实验，因为先前在临床前偏头痛模型中表明了其对于CPP的效力(De Felice等，Annals of neurology 2013;74(2):257-265)。在条件化前那天(mCHI之后第6天)，将假手术大鼠或mCHI大鼠放置 CPP盒中进入这两个室10分钟的时期，并记录在每个室中消耗的时间。室基于视觉(墙壁)和触觉(地板)线索区分。在条件化那天(mCHI之后第7天)早晨，动物接受载剂对照(i.p.盐水)，并且在2小时之后将其限制在载剂配对室中20分钟。下午，在载剂注射后4小时，动物接受舒马普坦，并在2小时后将其限制在相对室(舒马普坦配对室)中20分钟。由于数据表明最佳舒马普坦效力在施用之后2小时(Tfelt-Hansen等， Headache 1998;38(10):748-755)，所以在舒马普坦施用和室限制之间选择2 小时时期。24小时之后(mCHI之后第8天)将动物放入CPP设备中自由进入这两个室15分钟，并记录在每个室中消耗的时间。

[0903] GTN诱导的机械超敏性

[0904] 在mCHI之后第15天和第30天施用低剂量GTN(100μg/kg)以研究在mCHI之后2周头部疼痛超敏性消退之后，mCHI动物中是否存在对常见头痛触发物的任何提高的易感性。在GTN施用之后1小时和4小时如上评估了颅骨膜和后爪机械超敏性的发生。研究了急性施用舒马普坦或使用鼠mAb长期阻断CGRP作用减弱GTN诱导的疼痛超敏性的能力。

[0905] GTN诱导的条件化位置厌恶

[0906] 如上所述使用两个CPP盒测试对于GTN的条件化位置厌恶。该方案包括条件化前那天(mCHI之后第13天)，随后是条件化那天(第14天) 和24小时后的条件化后那天。在条件化那天的早晨，在GTN施用之前将动物首先限制在一个室中20分钟(GTN前配对室)。然后向动物施用GTN，且4小时后将其限制在相对室(GTN配对室)中20分钟。在条件化后那天，动物再次可自由进入这两个室。通过计算用鼠抗CGRP mAb或对照 IgG处理的动物在条件化前那天和条件化后那天在不同室中消耗的时间之间的差异评分来确定对GTN的厌恶。

[0907] 药物

[0908] 将舒马普坦(Sigma，USA)新鲜溶解于0.9％盐水中并以1mg/kg的剂量以1ml/kg的体积腹膜内(i.p.)施用。所有行为测试在舒马普坦施用之后2小时进行。药物剂量和施用时间基于药物的药动学以及内部试验工作和证明其在三叉神经痛动物模型中的效力的公开的研究(Oshinsky等， Headache 2012;52(9):1336-1349;Winner等，Mayo Clin Proc 2003;78(10):1214-1222)。靶向CGRP的鼠特异性mAb及其对照IgG由Teva Pharmaceuticals提供，并以30mg/kg的剂量和0.54ml/100g的体积i.p.施用(Kopruszinski等，Cephalalgia: an International Journal of Headache 2016)。第一次施用在mCHI诱导之后立即递送，

并随后每6天进行。将 GTN(American Reagent,USA)新鲜溶解于0.9％盐水中,并以100μg/kg 的剂量和1ml/kg的体积腹膜内(i.p.)施用。GTN的最终载剂浓度为0.6％丙二醇、0.6％乙醇和0.9％盐水。先前的工作已经显示利用6％丙二醇和乙醇载剂浓度对机械阈值没有影响(Pradhan等,Pain 2014;155(2):269-274)。

[0909] 数据分析

[0910] 使用Statview(SAS Institute,New York,NY,USA)进行统计学分析。分别使用Shapiro-Wilk和Levene检验评估正态性和方差齐性。使用双因素重复测量方差分析(ANOVA)来确定时间、头部创伤和药物治疗或其相互作用对空旷场地中的自主活动或者使用冯弗雷装置的颅骨膜超敏性的发生的主要影响。适当时,使用Fisher's LSD事后检验或不成对的双尾 t检验来评估组之间和时间点之间的差异。数据表示为平均值±平均值的标准误差。$p<0.05$被认为是统计学上显著性的。

[0911] 结果

[0912] mCHI/经震荡动物中垂直竖起活动降低但没有认知缺陷的证据

[0913] 在mCHI之后,动物在空旷场地中表现出降低的垂直探究(竖起)活动(RM ANOVA时间:$F_{(4,56)}=7.03$,$p<0.01$,损伤$F_{(1,14)}=21.31$,$p<0.01$)。不成对的双尾t检验显示,在mCHI之后48小时、72小时和第7天,与假手术组相比,mCHI组表现出持续降低的垂直探究活动($p<0.01$)(图 12A),表明轻度创伤性脑损伤。然而,在这些时间点,在总行进距离、中央区域探究或趋触性(thigmotaxis)方面未检测到变化(所有$p>0.05$)。如通过新物体识别测试测量的,在损伤之后7天在mCHI动物中也没有重大的认知缺陷的证据($p>0.05$)(图12B)。

[0914] mCHI动物中触觉超敏性的发生

[0915] 使用冯弗雷细丝,在mCHI中鉴定了头部触觉超敏性的时间依赖性发生,但在假手术动物中未鉴定出。重复测量ANOVA揭示了时间($F_{(4,48)}=7.54$,$p<0.001$)和时间x损伤相互作用($F_{(4,48)}=5.71$,$p<0.001$)的显著影响。不成对的双尾t检验显示与假手术组相比,在72小时和第7天时 mCHI组中的响应阈值显著降低且伤害感受评分显著提高(二者$p<0.05$)(图13A和13C)。在任何测试的时间点,mCHI未导致后爪机械超敏性的发生(图13B和13D)。

[0916] 急性舒马普坦处理和长期CGRP阻滞对mCHI诱导的头触觉超敏性的影响

[0917] 总体而言,CHI之后72h的急性舒马普坦处理减弱了头部触觉超敏性。(单因素ANOVA $F_{(2,17)}=8.79$,$p<0.05$)(图14A和14B)。在mCHI之后立即以及随后每6天开始,测试使用阻断性mAb或对照IgG的CGRP 的长期阻断发生mCHI相关头部触觉超敏性的能力。总体而言,CGRP阻断减弱了mCHI诱导的机械超敏性(RM ANOVA时间:$F_{(3,39)}=17.76$, $p<0.001$)。不成对的双尾t检验表明,在mCHI之后第7天,与对照IgG 处理组相比,抗CGRP mAb处理组具有显著提高的响应阈值和降低的伤害感受评分(图14C和14D,$p<0.05$)。用抗CGRP mAb的长期处理对假手术动物的机械响应阈值没有任何影响,这与Kopruszinski等的当前研究结果一致,他们也使用了抗CGRP mAb(Kopruszinski等,Cephalalgia:an International Journal of Headache 2016)。

[0918] mCHI动物中舒马普坦相关条件化位置偏好

[0919] 为了研究mCHI动物中PTH疼痛的厌恶性质,使用CPP范例来确定头部损伤之后7天,抗偏头痛药物舒马普坦是否可在mCHI大鼠中产生 CPP,但不能在假手术对照中产生。单个大鼠的差异评分(每个室的条件化后-条件化前时间差)表明,与假手术对照相比,mCHI大鼠

表现出显著提高的在舒马普坦室中的时间(p＜0.01)，表明全身性舒马普坦处理缓解了mCHI的厌恶性、头痛样效应。

[0920] 向无症状mCHI动物施用GTN之后机械超敏性的发生

[0921] 在mCHI之后第14天，假手术组与mCHI组之间不存在头部触觉敏感性的显著性差异。为了确定经受mCHI的大鼠在急性行为症状消退之后是否对偏头痛触发事件发生增强的触觉疼痛敏感性，我们在损伤之后第 15天和30天施用常见的偏头痛触发剂GTN之后，测试了对于机械刺激的头部和头外响应的变化。在第15天，GTN的施用导致mCHI动物中恢复的且明显的头部机械超敏性(RM ANOVA时间:$F_{(1,14)}$＝38.68，p＜0.001，时间x GTN处理$F_{(2,14)}$＝5.18，p＜0.05)，但在假手术动物中没有。Fisher′s LSD事后检验显示，与假手术对照相比，mCHI组在GTN之后1小时 (p＜0.01)和4小时(p＜0.001)表现出显著降低的头部响应阈值和提高的伤害感受评分(图16A和16B)。在mCHI之后第30天，GTN也产生明显的头部超敏性(RM ANOVA处理:$F_{(1,13)}$＝9.7，p＜0.05，时间x GTN处理: $F_{(2,26)}$＝3.24，p＜0.05)。Fisher′s LSD事后检验显示与阳性对照相比，mCHI 组在GTN之后1小时和4小时显示显著降低的头部响应阈值(p＜0.05)，以及在GTN之后4小时提高的伤害感受评分(图16C和16D，p＜0.05)。

[0922] 在损伤之后第15天(RM ANOVA时间:$F_{(2,26)}$＝8.27，p＜0.01，和GTN 处理:$F_{(1,14)}$＝8.12，p＜0.05)和第30天(RM ANOVA时间:$F_{(2,26)}$＝37.04， p＜0.05，和GTN处理:$F_{(2,26)}$＝3.24，p＜0.05)，GTN施用也在mCHI动物中选择性诱导了后爪机械超敏性，然而其限于降低的机械阈值。Fisher′s LSD 事后检验显示与假手术对照相比，mCHI组在第15天GTN之后4小时 (p＜0.05)和第30天GTN之后1小时显示出显著降低的机械阈值(图17A 和17C，p＜0.05)。

[0923] 急性舒马普坦处理和长期CGRP阻断对mCHI动物中GTN诱发的机械超敏性的影响

[0924] 在mCHI之后第15天，当在GTN之后1小时施用时，急性舒马普坦处理减弱了GTN引起的延迟性机械头部超敏性(在4小时时)。不成对的双尾t检验显示，与载剂处理的动物相比，急性舒马普坦处理显著降低了响应阈值的下降和伤害感受评分的提高(p＜0.001和p＜0.05，图18A和 18B)。舒马普坦处理并未减弱mCHI动物中GTN诱发的后爪超敏性。在 mCHI之后第15天，用抗CGRP mAb的长期处理阻止了GTN诱导的机械超敏性。不成对的双尾t检验显示，与对照IgG组相比，抗CGRP mAb 组显示出显著提高的响应阈值和降低的伤害感受评分(分别为p＜0.05，p ＜0.01，图18C和18D)。在损伤之后第30天，当与用对照IgG的处理相比时，抗CGRP mAb处理还有效减弱了GTN诱导的机械阈值降低(p ＜0.05，不成对的双尾t检验)。然而，抗CGRP mAb处理在阻断mCHI动物中GTN诱发的后爪超敏性中是无效的。

[0925] 对于GNT的条件化位置厌恶

[0926] 使用条件化位置厌恶范例，对mCHI之后的GTN施用进行测试，以判定其是否可诱导响应于抗CGRP mAb处理的持续疼痛样行为。在mCHI 之后第14天向mCHI动物施用GTN导致在用对照IgG处理的动物中的条件化位置厌恶，其为GTN配对室中降低的时间，表明在头部疼痛超敏性已经消退时mCHI动物中GTN诱发的疼痛。在用抗CGRP mAb处理的 mCHI动物中没有类似的GTN诱发的条件化位置厌恶的证据(图19A和 19B)，表明mCHI动物中GTN诱发的疼痛是CGRP依赖性的。

[0927] 抗CGRP mAb的长期处理对PTH相关行为的影响

[0928] 本研究的主要研究结果如下:1)通过mCHI的震荡与降低的竖起(即，探究性活动)

的急性期相关，但与主要运动或认知缺陷无关，表明轻度暂时形式的创伤性脑损伤(Kilboume等，J Neurotrauma 2009;26(12)：2233-2243)，2)经震荡的动物发生了头部触觉疼痛超敏性，其在损伤之后2周消退，并且其通过用舒马普坦的急性处理和通过阻断性mAb对 CGRP的延长的抑制而减弱，3)当使用全身性舒马普坦处理作为镇痛处理时，经历mCHI的动物在CPP范例中显示自发/持续疼痛样行为，以及 4)在头痛/疼痛行为消退后，施用头痛触发物GTN导致了新的和明显的头部超敏性以及被舒马普坦和抗CGRP mAb处理抑制的条件化位置厌恶。

[0929] 目前，对于PTH管理没有基于证据的治疗指南，因此通常将其类似于其类似的原发性头痛疾病进行治疗，研究显示舒马普坦在一些具有偏头痛特征的PTH患者中的效力(Abend等，J Child Neurol 2008;23(4):438-440; Lucas，Curr Pain Headache Rep 2015;19(10):48)。然而，由于显著比例的患者对曲普坦治疗没有响应(Visser等，Headache 1996;36(8):471-475)，因此需要其他治疗选择。基于CGRP在偏头痛和头痛中的假设作用(Pietrobon 等，Annu Rev Physiol 2013;75:365-391;Russo，Annual review of pharmacology and toxicology 2015;55:533-552)，一种有希望的新的治疗策略使用阻断mAb靶向CGRP或其受体(Mitsikostas等，BMC Med 2015;13:279)。一种这样的mAb TEV-48125的IIb期试验已经表明这种药剂在安全性、有效性和耐受性方面的有利结果(Bigal等，Lancet Neurol 2015;14(11):1081-1090;Bigal等，Lancet Neurol 2015;14(11):1091-1100)。来自目前研究的结果表明，通过啮齿动物特异性中和mAb抑制CGRP可减轻由mCHI诱发的头痛/疼痛症状，这表明CGRP参与介导PTH疼痛的发生。用抗CGRP mAb的处理还有效减弱了mCHI动物中GTN相关的头部机械超敏性，并且减弱了对于GTN的条件化位置厌恶，指向CGRP也是头部创伤诱发的痛觉过敏致敏样作用的介质，特别是GTN引起的头痛样异常性疼痛和持续性疼痛。由于mAb不太可能穿过血脑屏障(由于其大分子量)，其在mCHI动物中的作用机制可能涉及外周部位，可能中断mCHI诱发的脑膜或骨膜炎性响应。由于抗CGRP mAb治疗对于改善GTN 诱发的后爪机械致敏的发生无效，我们提出在mCHI动物中的这种响应是中枢介导的。

[0930] 生物材料的保藏

[0931] 以下材料已经保藏于美国典型培养物保藏中心(American Type Culture Collection)，10801 University Boulevard，Manassas，Virginia 20110-2209，USA(ATCC)：

[0932]

材料	抗体编号	ATCC 登录号	保藏日期
pDb.CGRP.hFcGI	G1 重链	PTA-6867	2005 年 7 月 15 日
pEb.CGRP.hKGI	G1 轻链	PTA-6866	2005 年 7 月 15 日

[0933] 载体pEb.CGRP.hKGI是编码G1轻链可变区和轻链κ恒定区的多核苷酸；且载体pDb.CGRP.hFcGI是编码G1重链可变区和重链IgG2恒定区的多核苷酸，所述重链IgG2恒定区包含以下突变：A330P331至S330S331 (参照野生型IgG2序列进行氨基酸编号；参见Eur.J.Immunol.(1999) 29:2613-2624)。

[0934] 这些保藏是根据国际承认用于专利程序目的的微生物保存布达佩斯条约及其相关条例(布达佩斯条约)的条款进行的。这确保从保藏之日起维持保藏的活培养物30年。保

藏物可由ATCC根据布达佩斯条约的条款提供，并受Rinat Neuroscience Corp.与ATCC之间的协议的约束，该协议保证在相关美国专利发布或者任何美国或外国专利申请向公众公开(以在先的为准)之后，永久且无限制地向公众提供保藏培养物的后代，并且保证由按照35 USC Section 122授权的美国专利和商标局长和该局长据此的裁决(包括特别参照886 OG 638的37 CFR Section 1.14)确定者可获得所述后代。

[0935] 本申请的受让人同意，如果在合适的条件下培养时，保藏材料的培养物死亡或丢失或毁坏，一经得到通知便迅速用另一相同者来代替。保藏材料的可用性不应解释为是在违背任何政府的主管部门根据其专利法所授权的权利的前提下实施本发明的许可。

[0936] 抗体序列

[0937] G1重链可变区氨基酸序列(SEQ ID NO:1)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWISWVRQAPGKGLEWVAEIRSESDA

[0938] SATHYAEAVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCLAYFDYGLAIQNYWGQG TLVTVSS

[0939] G1轻链可变区氨基酸序列(SEQ ID NO:2)

[0940] EIVLTQSPATLSLSPGERATLSCKASKRVTTYVSWYQQKPGQAPRLLIYGASNRYLGIP ARFSGSGSGTDFTLTISSLEPEDFAVYYCSQSYNYPYTFGQGTKLEIK

[0941] G1 CDR H1(延伸的CDR)(SEQ ID NO:3)

[0942] GFTFSNYWIS

[0943] G1 CDR H2(延伸的CDR)(SEQ ID NO:4)

[0944] EIRSESDASATHYAEAVKG

[0945] G1 CDR H3(SEQ ID NO:5)

[0946] YFDYGLAIQNY

[0947] G1 CDR L1(SEQ ID NO:6)

[0948] KASKRVTTYVS

[0949] G1 CDR L2(SEQ ID NO:7)

[0950] GASNRYL

[0951] G1 CDR L3(SEQ ID NO:8)

[0952] SQSYNYPYT

[0953] G1重链可变区核苷酸序列(SEQ ID NO:9)

GAAGTTCAGCTGGTTGAATCCGGTGGTGGTCTGGTTCAGCCAGGTGGTTCCCTGC GTCTGTCCTGCGCTGCTTCCGGTTTCACCTTCTCCAACTACTGGATCTCCTGGGTT CGTCAGGCTCCTGGTAAAGGTCTGGAATGGGTTGCTGAAATCCGTTCCGAATCCG

[0954] ACGCGTCCGCTACCCATTACGCTGAAGCTGTTAAAGGTCGTTTCACCATCTCCCGT GACAACGCTAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGTGCTGAAGACAC CGCTGTTTACTACTGCCTGGCTTACTTTGACTACGGTCTGGCTATCCAGAACTACT GGGGTCAGGGTACCCTGGTTACCGTTTCCTCC

[0955] G1轻链可变区核苷酸序列(SEQ ID NO:10)

[0956] GAAATCGTTCTGACCCAGTCCCCGGCTACCCTGTCCCTGTCCCCAGGTGAACGTGCT ACCCTGTCCTGCAAAGCTTCCAAACGGGTTACCACCTACGTTTCCTGGTACCAGCAGA AACCCGGTCAGGCTCCTCGTCTGCTGATCTACGGTGCTTCCAACCGTTACCTCGGTAT CCCAGCTCGTTTCTCCGGTTCCGGTTCCGGTACCGACTTCACCCTGACCATCTCCTCC CTGGAACCCGAAGACTTCGCTGTTTACTACTGCAGTCAGTCCTACAACTACCCCTACA CCTTCGGTCAGGGTACCAAACTGGAAATCAAA

[0957] G1重链完整抗体氨基酸序列(包括如本文中所述的经修饰IgG2)(SEQ ID NO:11)

[0958] EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWISWVRQAPGKGLEWVAEIRSESDA SATHYAEAVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCLAYFDYGLAIQNYWGQG TLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPC PAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0959] G1轻链完整抗体氨基酸序列(SEQ ID NO:12)

[0960] EIVLTQSPATLSLSPGERATLSCKASKRVTTYVSWYQQKPGQAPRLLIYGASNRYLGIP ARFSGSGSGTDFTLTISSLEPEDFAVYYCSQSYNYPYTFGQGTKLEIKRTVAAPSVFIF PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0961] G1重链完整抗体核苷酸序列(包括如本文中所述的经修饰IgG2)(SEQ ID NO:13)

GAAGTTCAGCTGGTTGAATCCGGTGGTGGTCTGGTTCAGCCAGGTGGTTCCCTGC
GTCTGTCCTGCGCTGCTTCCGGTTTCACCTTCTCCAACTACTGGATCTCCTGGGTT
CGTCAGGCTCCTGGTAAAGGTCTGGAATGGGTTGCTGAAATCCGTTCCGAATCCG
ACGCGTCCGCTACCCATTACGCTGAAGCTGTTAAAGGTCGTTTCACCATCTCCCGT
GACAACGCTAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGTGCTGAAGACA
CCGCTGTTTACTACTGCCTGGCTTACTTTGACTACGGTCTGGCTATCCAGAACTAC
TGGGGTCAGGGTACCCTGGTTACCGTTTCCTCCGCCTCCACCAAGGGCCCATCTG
TCTTCCCACTGGCCCCATGCTCCCGCAGCACCTCCGAGAGCACAGCCGCCCTGG
GCTGCCTGGTCAAGGACTACTTCCCAGAACCTGTGACCGTGTCCTGGAACTCTGG
CGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGTCCTGCAGTCCTCAGGTCTC
TACTCCCTCAGCAGCGTGGTGACCGTGCCATCCAGCAACTTCGGCACCCAGACCT

[0962] ACACCTGCAACGTAGATCACAAGCCAAGCAACACCAAGGTCGACAAGACCGTGGA
GAGAAAGTGTTGTGTGGAGTGTCCACCTTGTCCAGCCCCTCCAGTGGCCGGACCA
TCCGTGTTCCTGTTCCCTCCAAAGCCAAAGGACACCCTGATGATCTCCAGAACCCC
AGAGGTGACCTGTGTGGTGGTGGACGTGTCCCACGAGGACCCAGAGGTGCAGTT
CAACTGGTATGTGGACGGAGTGGAGGTGCACAACGCCAAGACCAAGCCAAGAGA
GGAGCAGTTCAACTCCACCTTCAGAGTGGTGAGCGTGCTGACCGTGGTGCACCAG
GACTGGCTGAACGGAAAGGAGTATAAGTGTAAGGTGTCCAACAAGGGACTGCCAT
CCAGCATCGAGAAGACCATCTCCAAGACCAAGGGACAGCCAAGAGAGCCACAGGT
GTATACCCTGCCCCCATCCAGAGAGGAGATGACCAAGAACCAGGTGTCCCTGACC
TGTCTGGTGAAGGGATTCTATCCATCCGACATCGCCGTGGAGTGGGAGTCCAACG
GACAGCCAGAGAACAACTATAAGACCACCCCTCCAATGCTGGACTCCGACGGATC
CTTCTTCCTGTATTCCAAGCTGACCGTGGACAAGTCCAGATGGCAGCAGGGAAAC
GTGTTCTCTTGTTCCGTGATGCACGAGGCCCTGCACAACCACTATACCCAGAAGAG
CCTGTCCCTGTCTCCAGGAAAGTAA

[0963] G1轻链完整抗体核苷酸序列(SEQ ID NO:14)

[0964] GAAATCGTTCTGACCCAGTCCCCGGCTACCCTGTCCCTGTCCCCAGGTGAACGTG CTACCCTGTCCTGCAAAGCTTCCAAACGGGTTACCACCTACGTTTCCTGGTACCAG CAGAAACCCGGTCAGGCTCCTCGTCTGCTGATCTACGGTGCTTCCAACCGTTACCT CGGTATCCCAGCTCGTTTCTCCGGTTCCGGTTCCGGTACCGACTTCACCCTGACC ATCTCCTCCCTGGAACCCGAAGACTTCGCTGTTTACTACTGCAGTCAGTCCTACAA CTACCCCTACACCTTCGGTCAGGGTACCAAACTGGAAATCAAACGCACTGTGGCT GCACCATCTGTCTTCATCTTCCCTCCATCTGATGAGCAGTTGAAATCCGGAACTGC CTCTGTTGTGTGCCTGCTGAATAACTTCTATCCGCGCGAGGCCAAAGTACAGTGGA AGGTGGATAACGCCCTCCAATCCGGTAACTCCCAGGAGAGTGTCACAGAGCAGGA CAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACCCTGAGCAAAGCAGAC TACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGTTCTC CAGTCACAAAGAGCTTCAACCGCGGTGAGTGCTAA

[0965] 人和大鼠CGRP的氨基酸序列比较(人α-CGRP(SEQ ID NO:15);人β-CGRP(SEQ ID NO:43);大鼠α-CGRP(SEQ ID NO:41);和大鼠β-CGRP(SEQ ID NO:44)):

[0966] NH_2-ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-$CONH_2$ （人 α-CGRP）

NH_2-ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF-$CONH_2$ （人 β-CGRP）

NH_2-SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSEAF-$CONH_2$ （大鼠 α-CGRP）

NH_2-SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSKAF-$CONH_2$ （大鼠 β-CGRP）

[0967] 轻链可变区LCVR17氨基酸序列(SEQ ID NO:58)

[0968] DIQMTQSPSSLSASVGDRVTITCRASQDIDNYLNWYQQKPGKAPKLLIYYTSEYHSGV PSRFSGSGSGTDFTFTISSLQPEDIATYYCQQGDALPPTFGQGTKLEIK

[0969] 重链可变区HCVR22氨基酸序列(SEQ ID NO:59)

[0970] QVQLVQSGAEVKKPGASVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT GDTRYIQKFAGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARLSDYVSGFSYWGQG TLVTVSS

[0971] 轻链可变区LCVR18氨基酸序列(SEQ ID NO:60)

[0972] DIQMTQSPSSLSASVGDRVTITCRASQDIDNYLNWYQQKPGKAPKLLIYYTSEYHSGV PSRFSGSGSGTDFTFTISSLQPEDIATYYCQQGDALPPTFGQGTKLEIK

[0973] 重链可变区HCVR23氨基酸序列(SEQ ID NO:61)

[0974] QVQLVQSGAEVKKPGASVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT GKTVYIQKFAGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARLSDYVSGFSYWGQG TLVTVSS

[0975] 轻链可变区LCVR19氨基酸序列(SEQ ID NO:62)

[0976] DIQMTQSPSSLSASVGDRVTITCRASKDISKYLNWYQQKPGKAPKLLIYYTSGYHSGVP SRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDALPPTFGGGTKVEIK

[0977] 重链可变区HCVR24氨基酸序列(SEQ ID NO:63)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT

[0978] GKTVYIQKFADRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQGT TVTVSS

[0979] 轻链可变区LCVR20氨基酸序列(SEQ ID NO:64)

[0980] DIQMTQSPSSLSASVGDRVTITCRASRPIDKYLNWYQQKPGKAPKLLIYYTSEYHSGVP SRFSGSGSGTDFTFTISSLQPEDIATYYCQQGDALPPTFGQGTKLEIK

[0981] 重链可变区HCVR25氨基酸序列(SEQ ID NO:65)

QVQLVQSGAEVKKPGASVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT

[0982] GKTVYIQKFAGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQG TLVTVSS

[0983] 轻链可变区LCVR21氨基酸序列(SEQ ID NO:66)

[0984] DIQMTQSPSSLSASVGDRVTITCRASQDIDKYLNWYQQKPGKAPKLLIYYTSGYHSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQGDALPPTFGGGTKVEIK

[0985] 重链可变区HCVR26氨基酸序列(SEQ ID NO:67)

QVQLVQSGAEVKKPGSSVKVSCKASGYTFGNYWMQWVRQAPGQGLEWMGAIYEGT

[0986] GKTVYIQKFAGRVTITADKSTSTAYMELSSLRSEDTAVYYCARLSDYVSGFGYWGQGT TVTVSS

[0987] 轻链可变区LCVR27氨基酸序列(SEQ ID NO:68)

[0988] QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASG VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIKR

[0989] 重链可变区HCVR28氨基酸序列(SEQ ID NO:69)

[0990] EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGAT YYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS

[0991] 轻链可变区LCVR29氨基酸序列(SEQ ID NO:70)

[0992] QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTLASG VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSSGDCFVFGGGTKVEIKR

[0993] 重链可变区HCVR30氨基酸序列(SEQ ID NO:71)

[0994] EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGINDN TYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS

[0995] 轻链可变区LCVR31氨基酸序列(SEQ ID NO:72)

[0996] QVLTQSPSSLSASVGDRVTINCQASQSVYDNNYLAWYQQKPGKVPKQLIYSTSTLASG VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCSSGDCFVFGGGTKVEIKR

[0997] 重链可变区HCVR32氨基酸序列(SEQ ID NO:73)

[0998] EVQLVESGGGLVQPGGSLRLSCAVSGLDLSSYYMQWVRQAPGKGLEWVGVIGINDN TYYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS

[0999] 轻链可变区LCVR33氨基酸序列(SEQ ID NO:74)

[1000] QVLTQTPSPVSAAVGSTVTINCQASQSVYHNTYLAWYQQKPGQPPKQLIYDASTLASG VPSRFSGSGSGTQFTLTISGVQCNDAAAYYCLGSYDCTNGDCFVFGGGTEVVVKR

[1001] 重链可变区HCVR34氨基酸序列(SEQ ID NO:75)

[1002] QSLEESGGRLVTPGTPLTLTCSVSGIDLSGYYMNWVRQAPGKGLEWIGVIGINGATYY ASWAKGRFTISKTSSTTVDLKMTSLTTEDTATYFCARGDIWGPGTLVTVSS

[1003] 轻链可变区LCVR35氨基酸序列(SEQ ID NO:76)

[1004] QVLTQSPSSLSASVGDRVTINCQASQSVYHNTYLAWYQQKPGKVPKQLIYDASTLASG VPSRFSGSGSGTDFTLTISSLQPEDVATYYCLGSYDCTNGDCFVFGGGTKVEIKR

[1005] 重链可变区HCVR36氨基酸序列(SEQ ID NO:77)

[1006] EVQLVESGGGLVQPGGSLRLSCAVSGIDLSGYYMNWVRQAPGKGLEWVGVIGINGAT YYASWAKGRFTISRDNSKTTVYLQMNSLRAEDTAVYFCARGDIWGQGTLVTVSS

[1007] 轻链可变区LCVR37氨基酸序列(SEQ ID NO:78)

[1008] QSVLTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKRPSG IPDRFSGSKSGTSTTLGITGLQTGDEADYYCGTWDSRLSAVVFGGGTKLTVL

[1009] 重链可变区HCVR38氨基酸序列(SEQ ID NO:79)

[1010] QVQLVESGGGVVQPGRSLRLSCAASGFTFSSFGMHWVRQAPGKGLEWVAVISFDGS IKYSVDSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCARDRLNYYDSSGYYHYKY YGMAVWGQGTTVTVSS

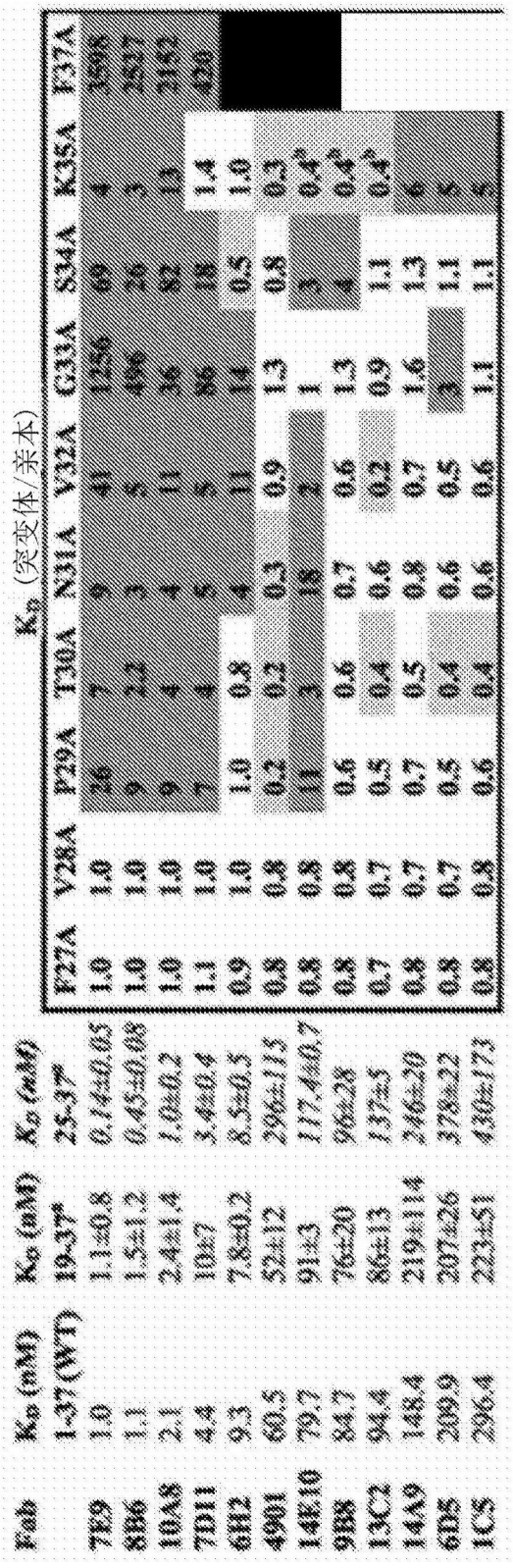

Fab
K_D (nM)
1-37 (WT)
K_D (nM)
19-37*
K_D (nM)
25-37*
K_D (突变体/亲本)
F27A
V28A
P29A
T30A
N31A
Y32A
G33A
S34A
K35A
F37A
7E9 1.0 1.1±0.8 0.14±0.05 1.0 1.0 26 7 9 41 1256 69 4 1506
8B6 1.1 1.5±1.2 0.45±0.08 1.0 1.0 9 2.2 3 5 496 26 3 2827
10A8 2.1 2.4±1.4 1.0±0.2 1.0 1.0 9 4 4 5 36 82 13 2152
7D11 4.4 10±7 3.4±0.4 1.1 1.0 7 4 5 5 86 18 1.4 420
6H2 9.3 7.8±0.2 8.5±0.5 0.9 1.0 1.0 0.8 4 11 14 0.5 1.0
4901 60.5 52±12 296±115 0.8 0.8 0.2 0.2 0.3 0.9 1.3 0.8 0.3
14E10 79.7 91±3 117.4±0.7 0.8 0.8 11 3 18 3 1 3 0.4*
9B8 84.7 76±20 96±28 0.8 0.8 0.6 0.6 0.7 0.6 1.3 4 0.4*
13C2 94.4 86±13 137±5 0.7 0.7 0.5 0.4 0.6 0.2 0.9 1.1 0.4*
14A9 148.4 219±114 246±20 0.8 0.7 0.7 0.5 0.8 0.7 1.6 1.3 6
6D5 209.9 207±26 378±22 0.8 0.7 0.5 0.4 0.6 0.5 3 1.1 3
1C5 296.4 223±51 430±173 0.8 0.8 0.6 0.4 0.6 0.6 1.1 1.1 4

图1

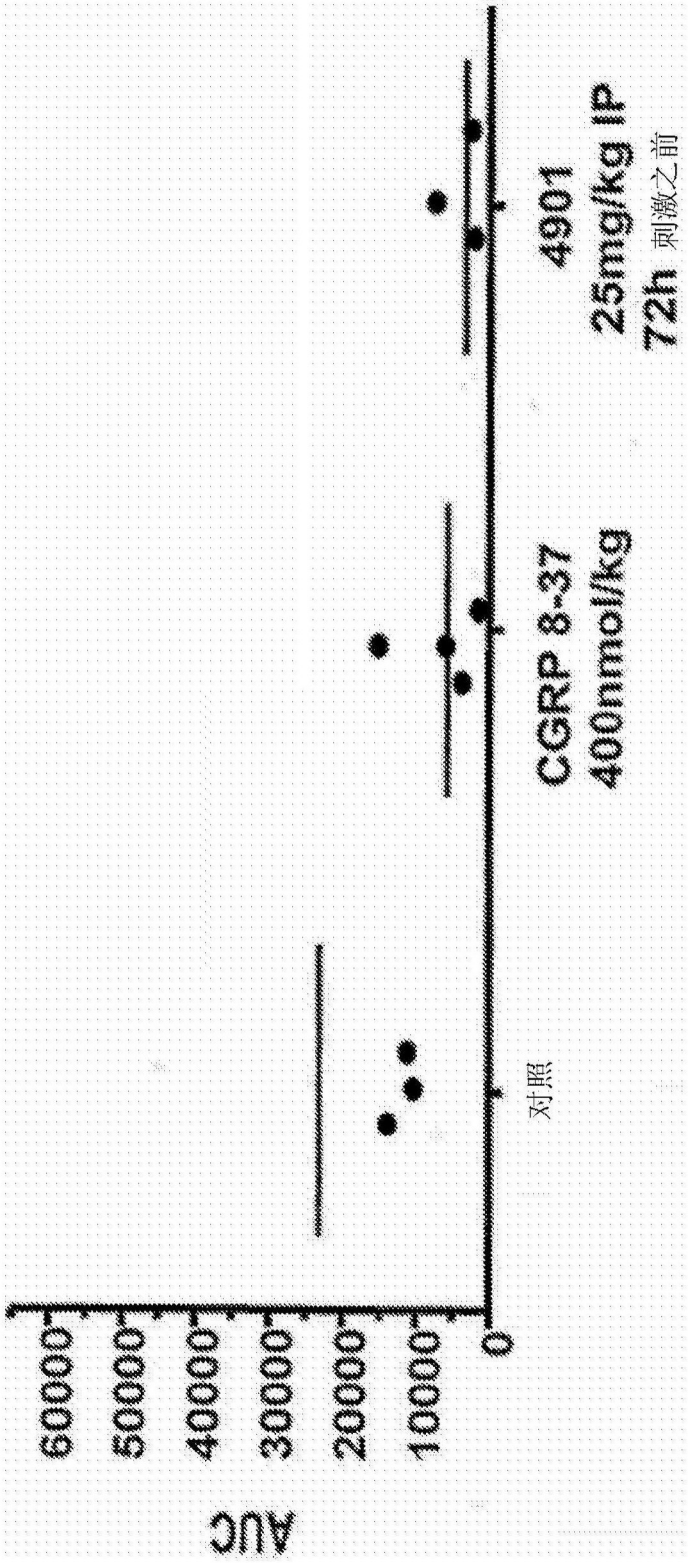

AUC
60000
50000
40000
30000
20000
10000
0
对照
CGRP 8-37
400nmol/kg
4901
25mg/kg IP
72h 刺激之前

图2A

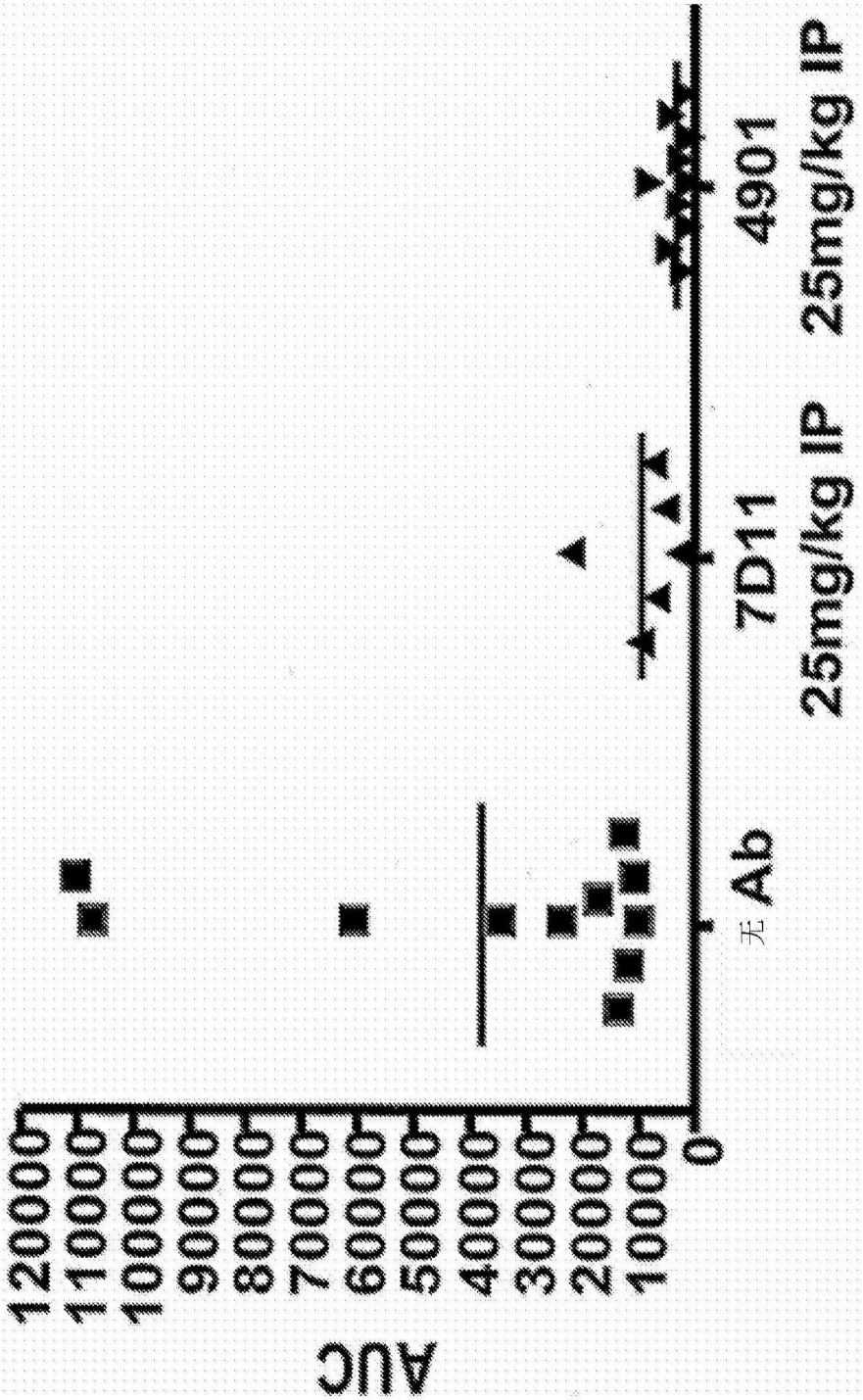

AUC
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
无 Ab
7D11
25mg/kg IP
4901
25mg/kg IP

图2B

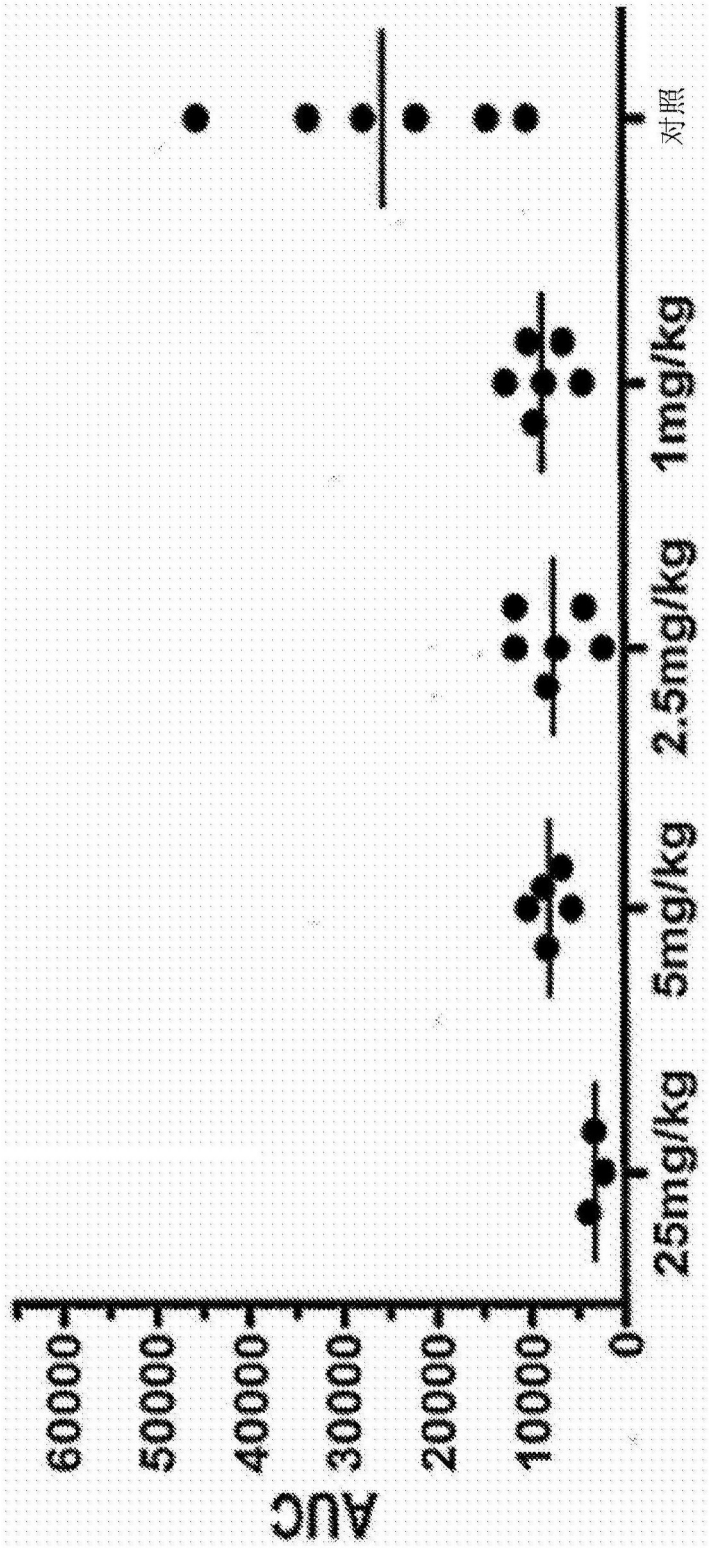

AUC
60000
50000
40000
30000
20000
10000
0
25mg/kg
5mg/kg
2.5mg/kg
1mg/kg
对照

图3

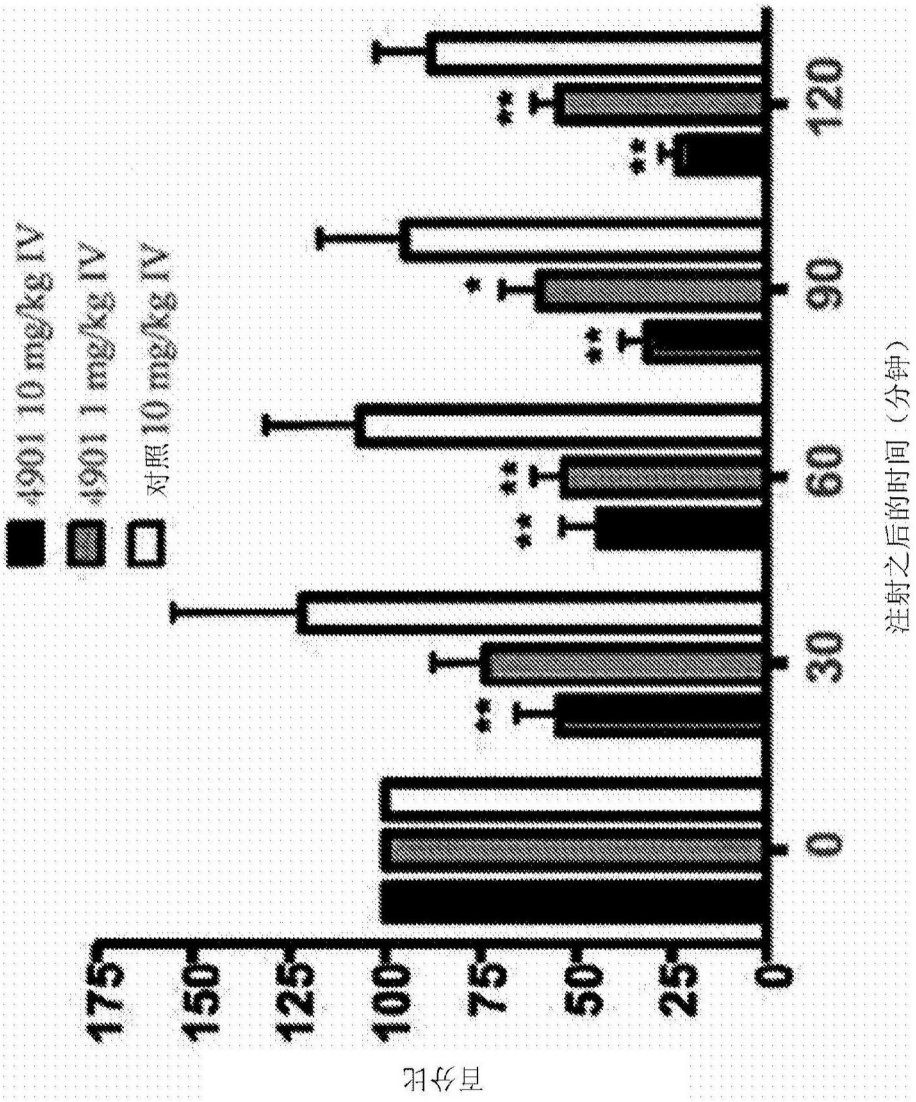

4901 10 mg/kg IV
4901 1 mg/kg IV
对照 10 mg/kg IV
注射之后的时间（分钟）
百分比
0
30
60
90
120
0
25
50
75
100
125
150
175

图4A

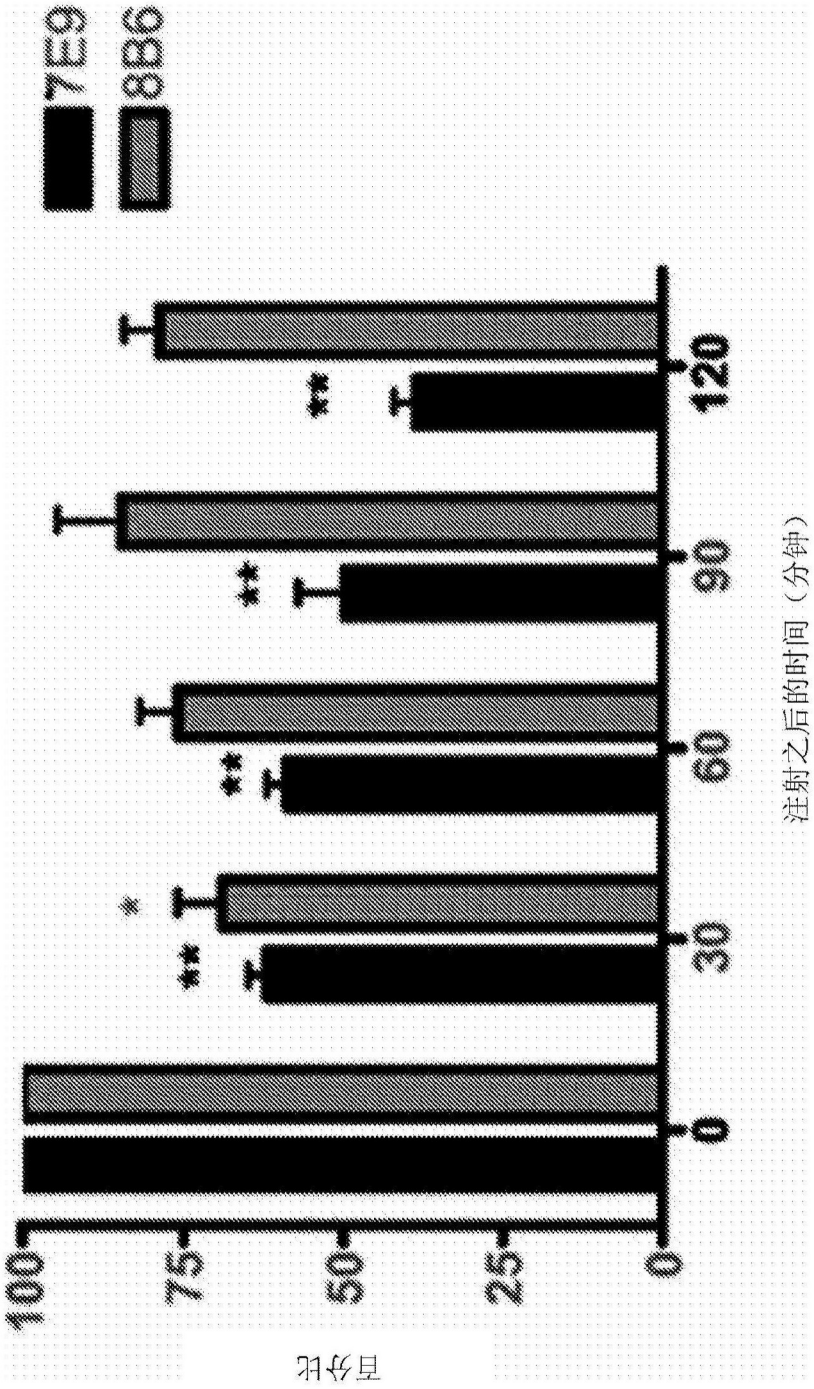


图4B

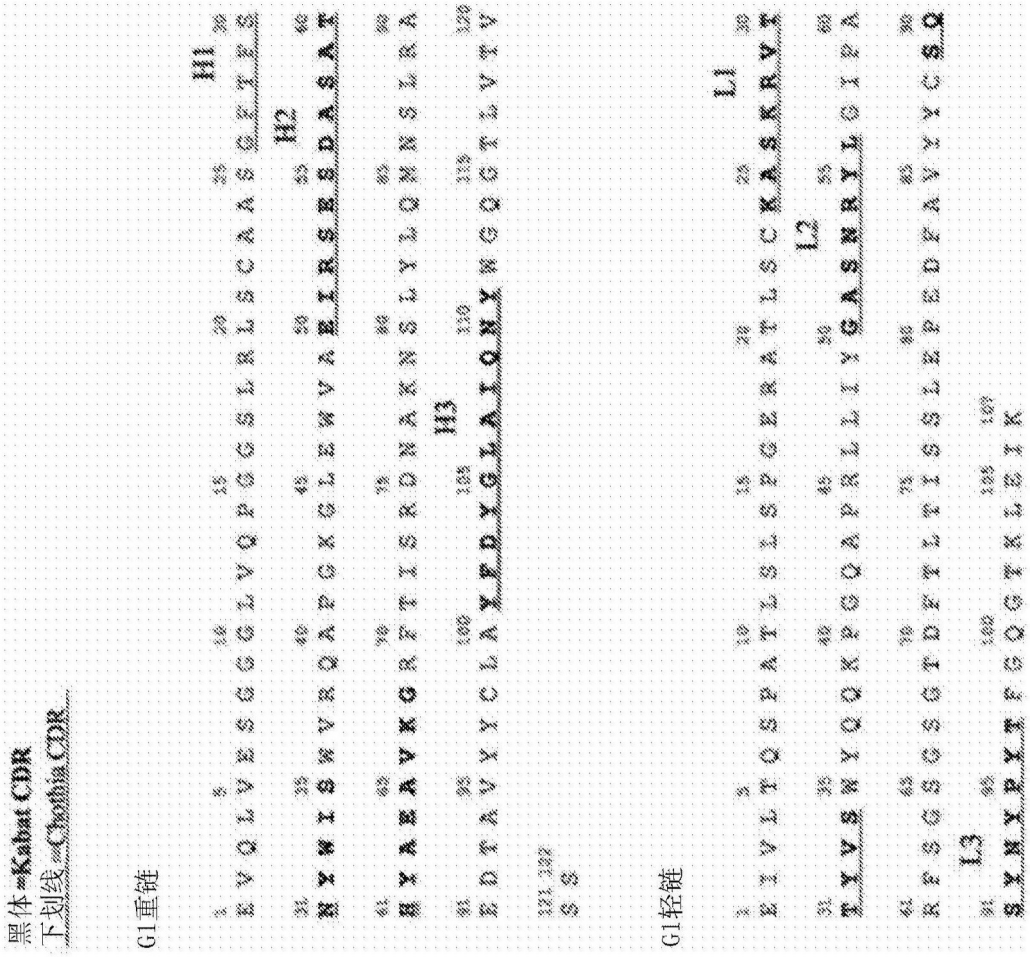


图5

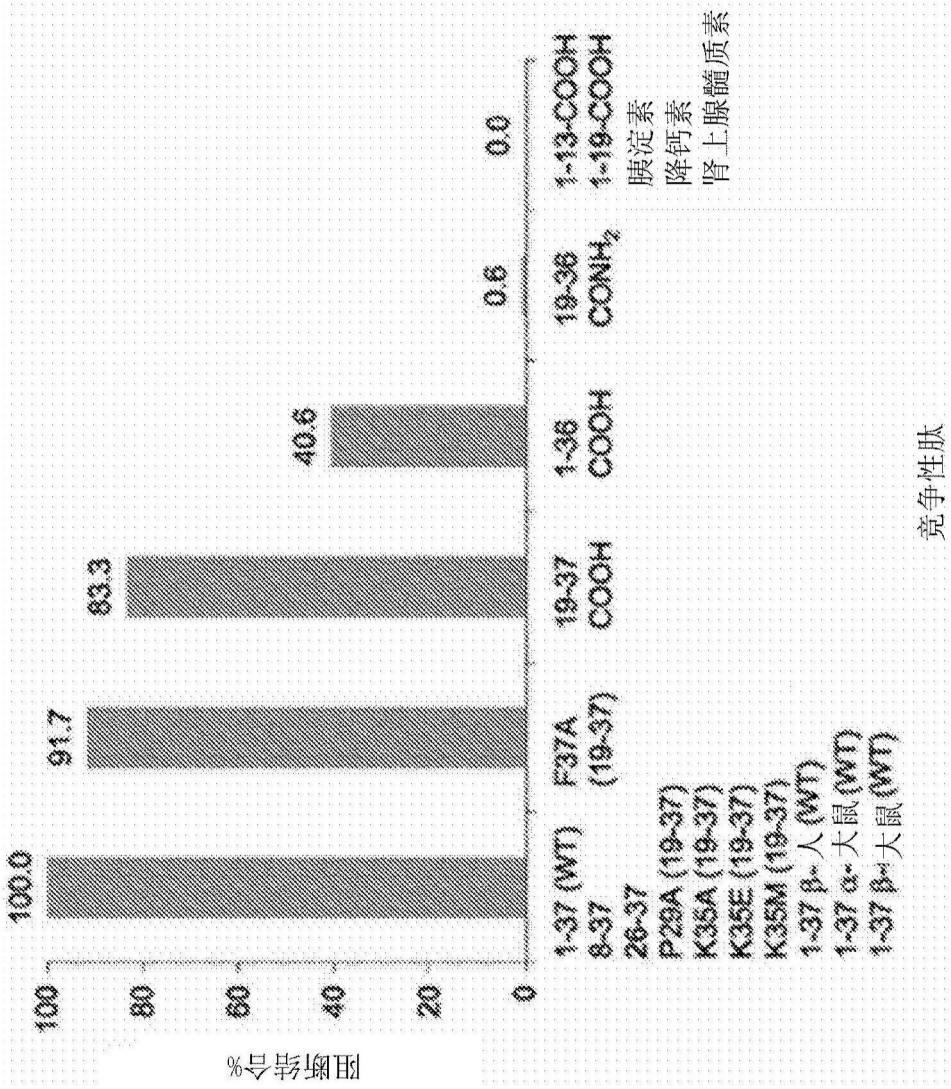

阻断结合%
100
80
60
40
20
0
100.0
91.7
83.3
40.6
0.6
0.0
1-37 (WT)
F37A (19-37)
19-37 COOH
1-36 COOH
19-38 CONH2
1-13-COOH
1-19-COOH
8-37
26-37
P29A (19-37)
K35A (19-37)
K35E (19-37)
K35M (19-37)
1-37 β-人 (WT)
1-37 α-大鼠 (WT)
1-37 β-大鼠 (WT)
胰淀素
降钙素
肾上腺髓质素
竞争性肽

图6

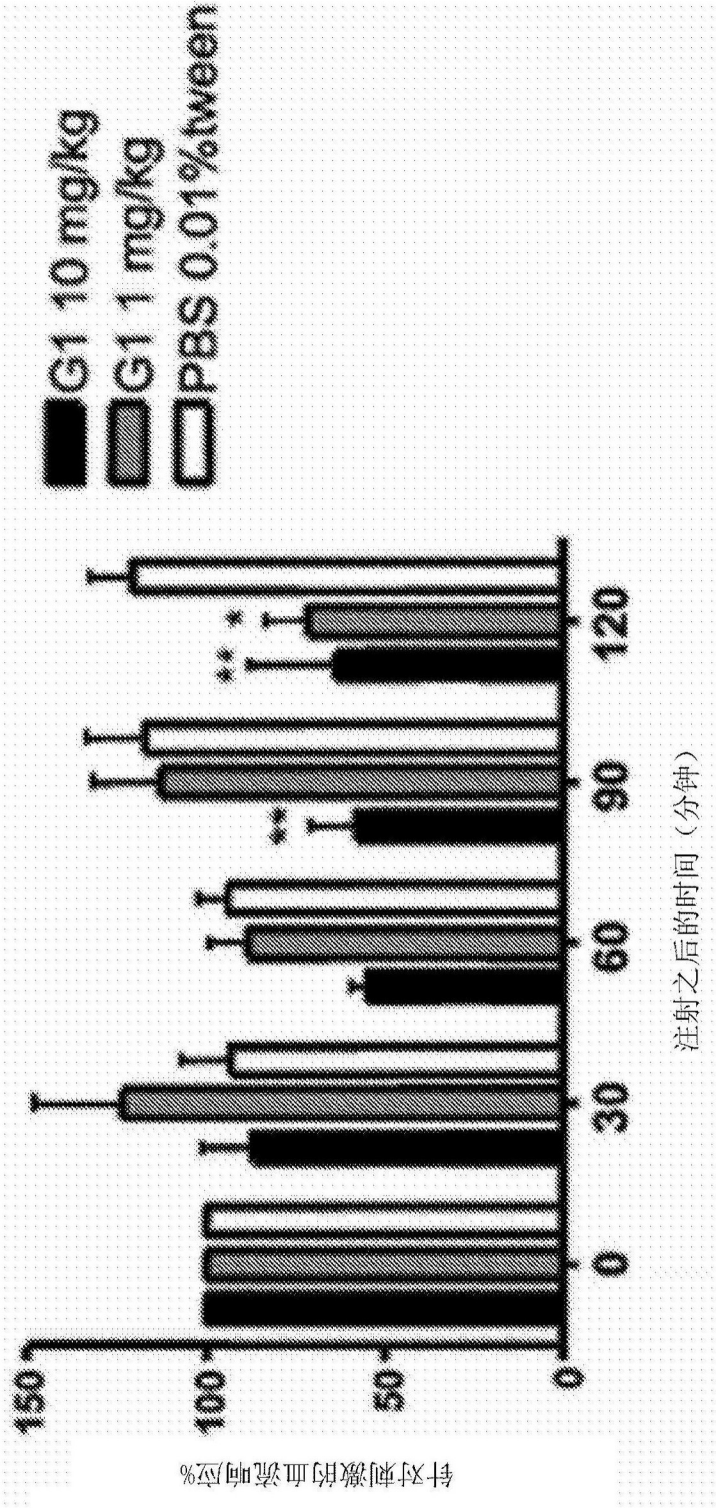

G1 10 mg/kg
G1 1 mg/kg
PBS 0.01%tween
针对刺激的血流响应%
150
100
50
0
0
30
60
90
120
注射之后的时间（分钟）
*
**
**

图7

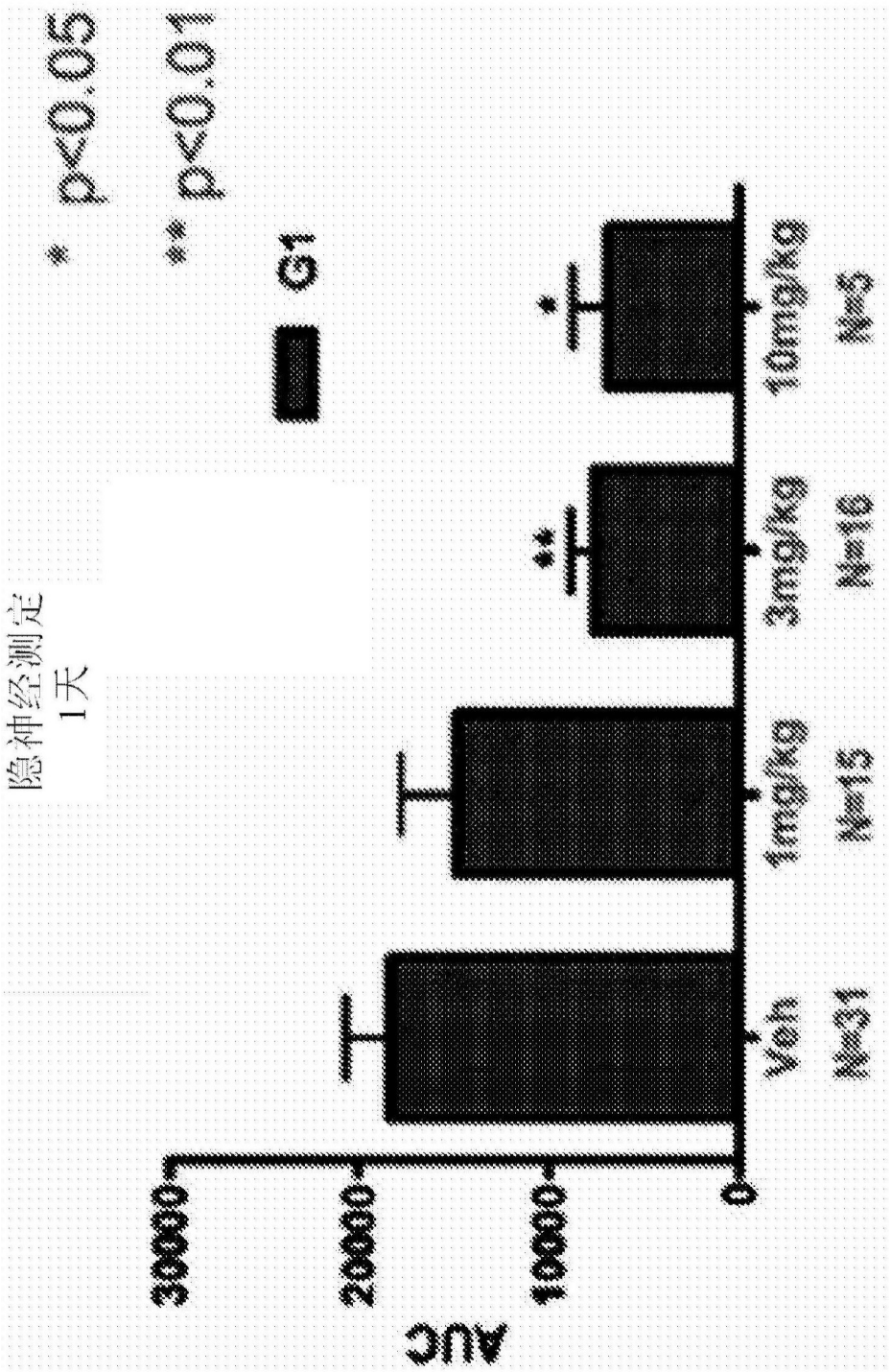

* p<0.05
** p<0.01
G1
隐神经测定
1天
AUC
30000
20000
10000
0
Veh
N=31
1mg/kg
N=15
3mg/kg
N=16
10mg/kg
N=3

图8A

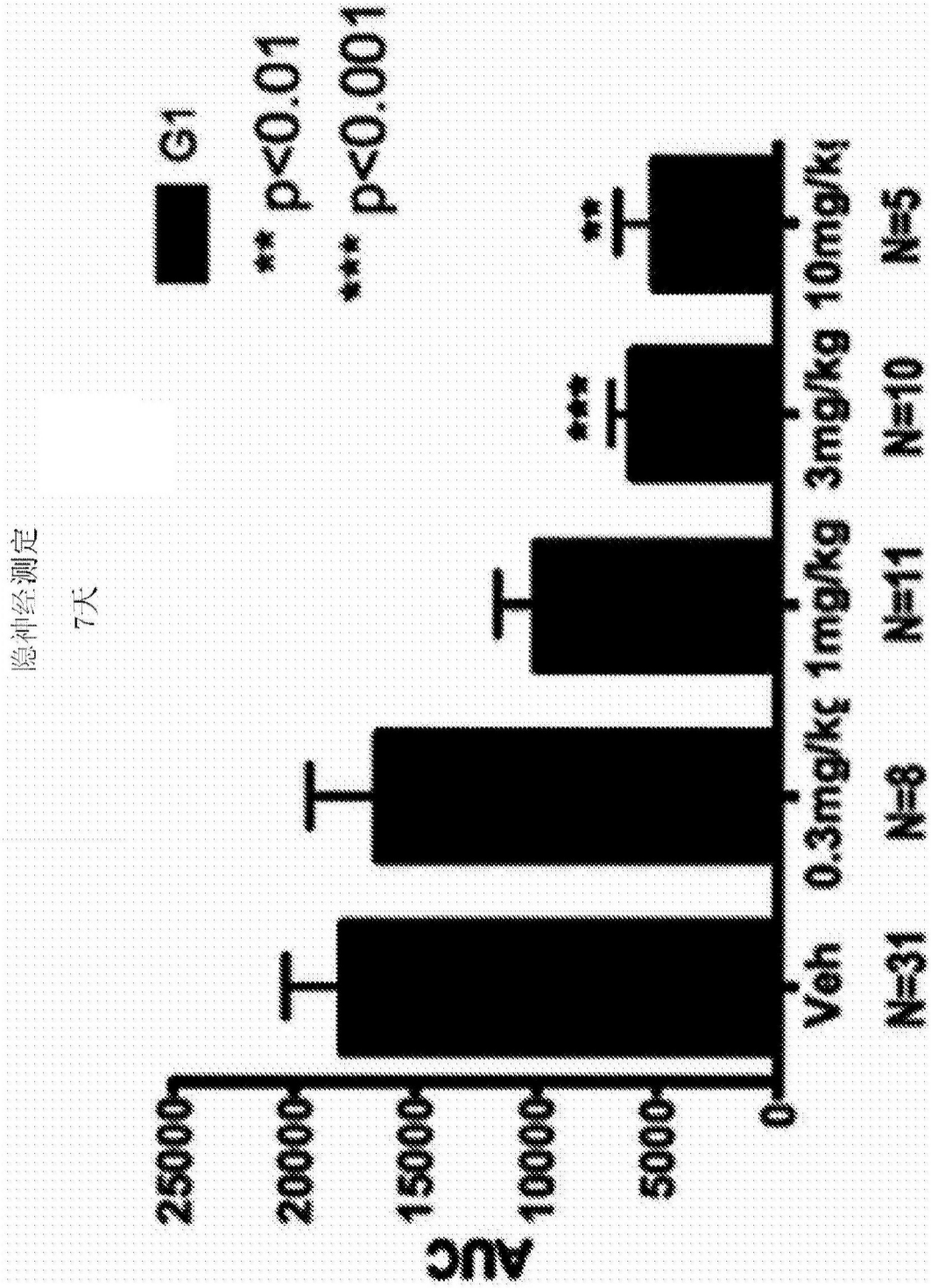

隐神经测定
7天
AUC
25000
20000
15000
10000
5000
0
G1
** p<0.01
*** p<0.001
Veh
0.3mg/kg
1mg/kg
3mg/kg
10mg/kg
N=31
N=8
N=11
N=10
N=5

图8B

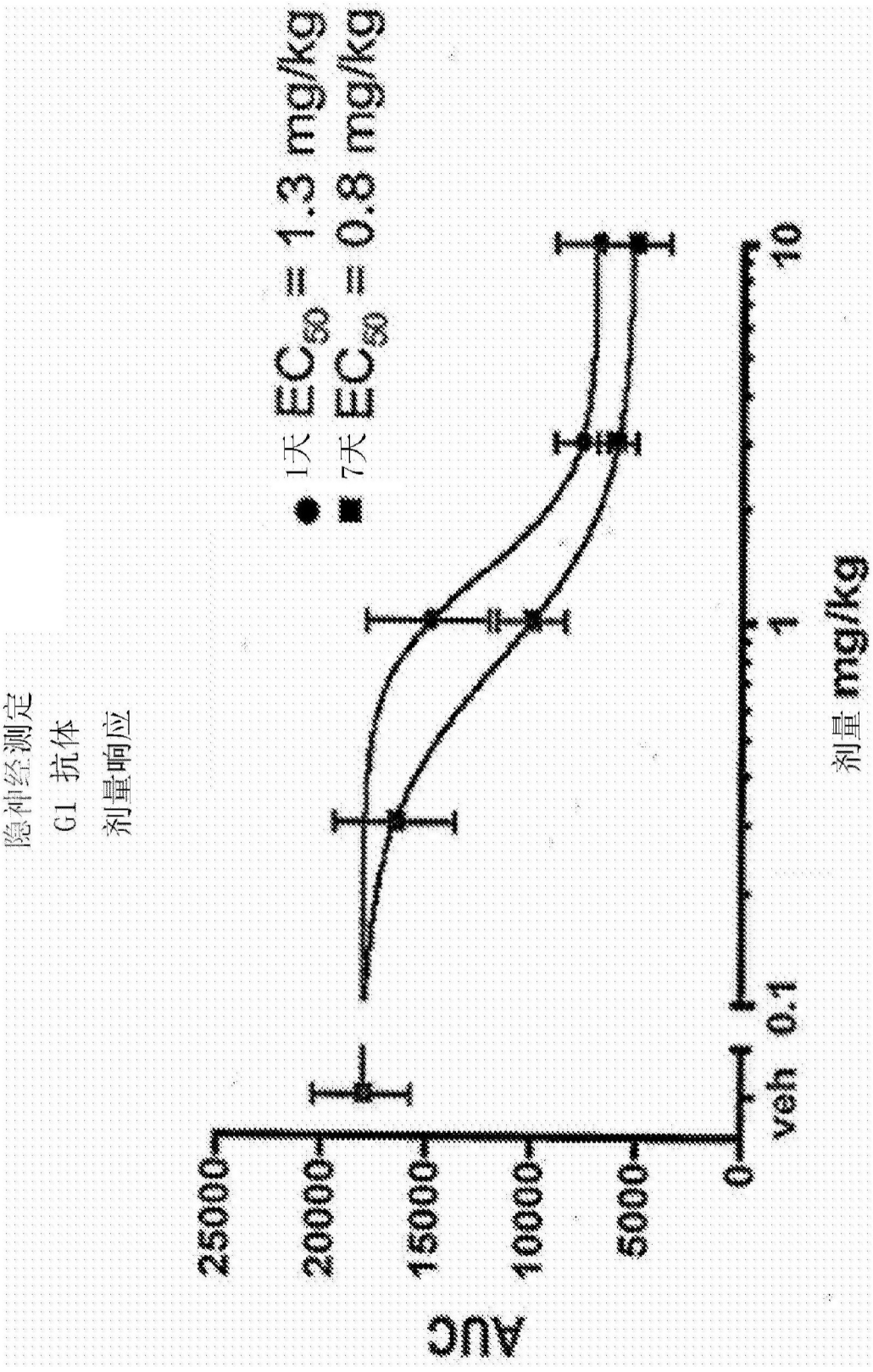

隐神经测定
G1 抗体
剂量响应
● 1天 EC_{50} = 1.3 mg/kg
■ 7天 EC_{50} = 0.8 mg/kg
AUC
25000
20000
15000
10000
5000
0
veh
0.1
1
10
剂量 mg/kg

图8C

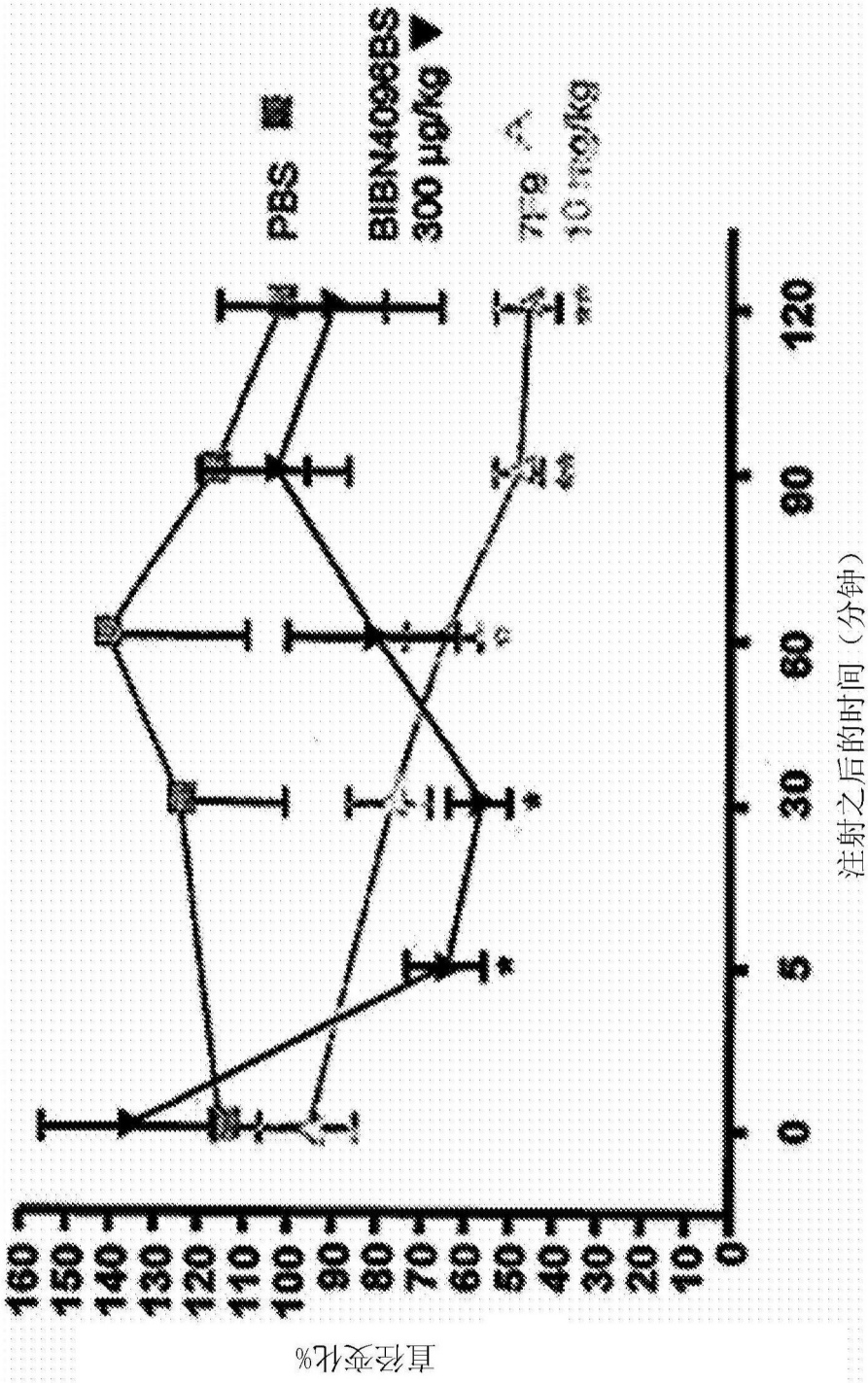

PBS
BIBN4096BS
300 μg/kg
ZD
10 mg/kg
直径变化%
注射之后的时间（分钟）
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
5
30
60
90
120

图9

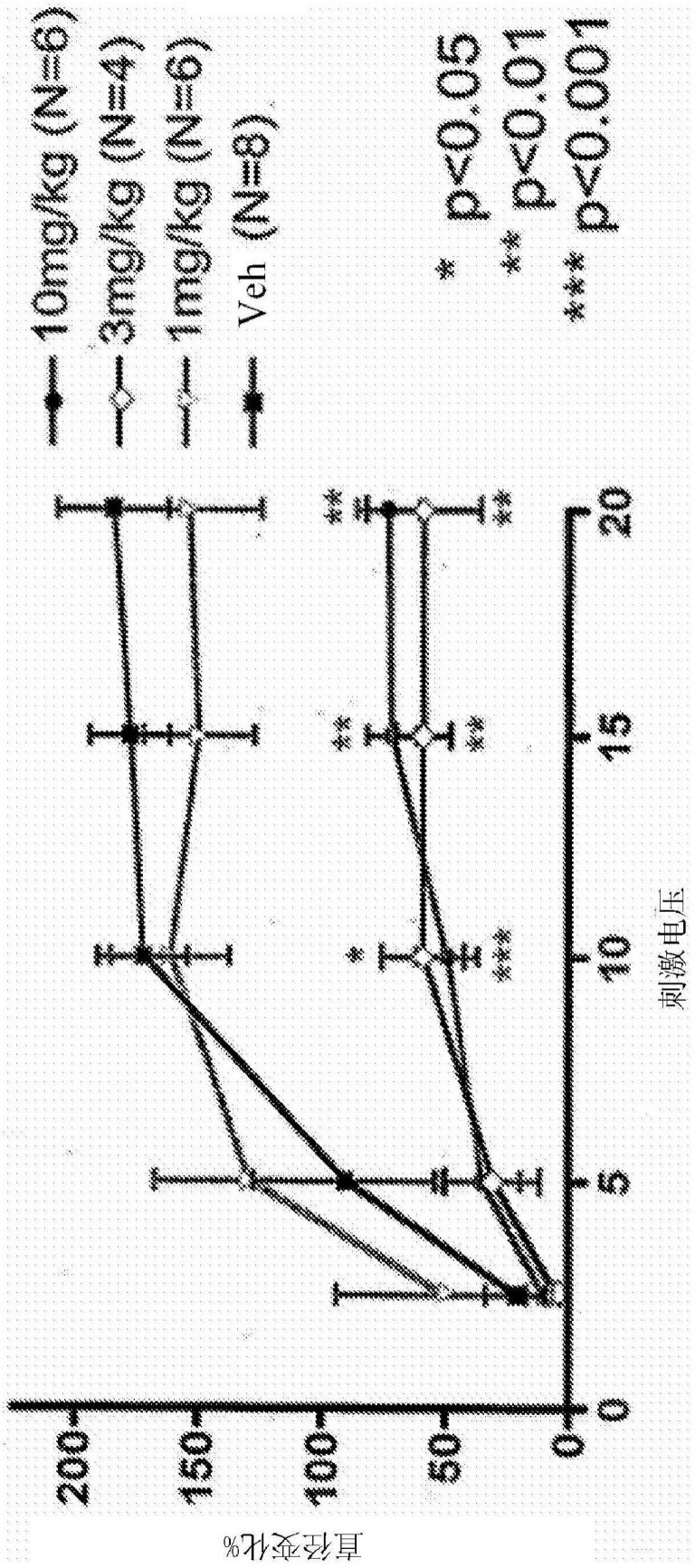

10mg/kg (N=6)
3mg/kg (N=4)
1mg/kg (N=6)
Veh (N=8)
* p<0.05
** p<0.01
*** p<0.001
直径变化%
刺激电压
200
150
100
50
0
0
5
10
15
20

图10

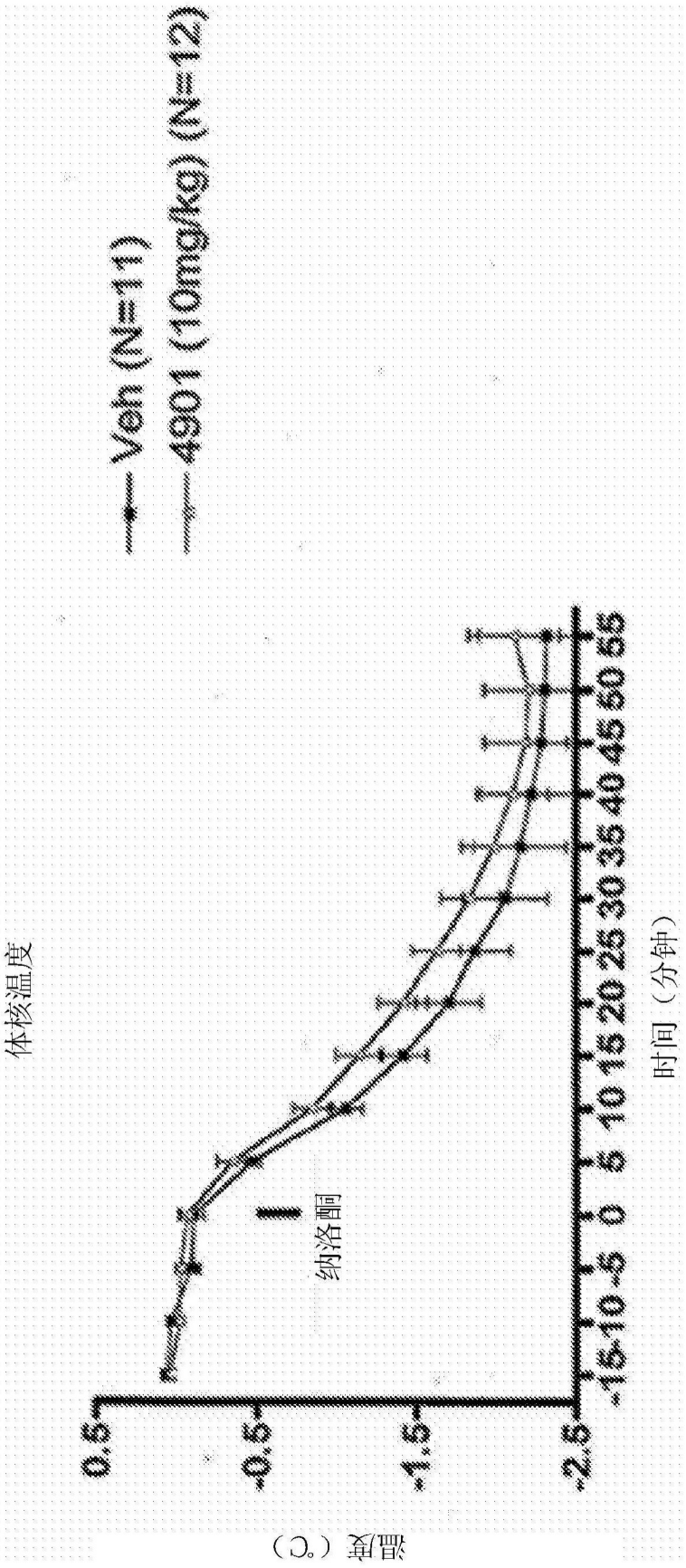

Veh (N=11)
4901 (10mg/kg) (N=12)
体核温度
纳洛酮
时间（分钟）
温度（°C）
0.5
-0.5
-1.5
-2.5
-16 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55 65

图11A

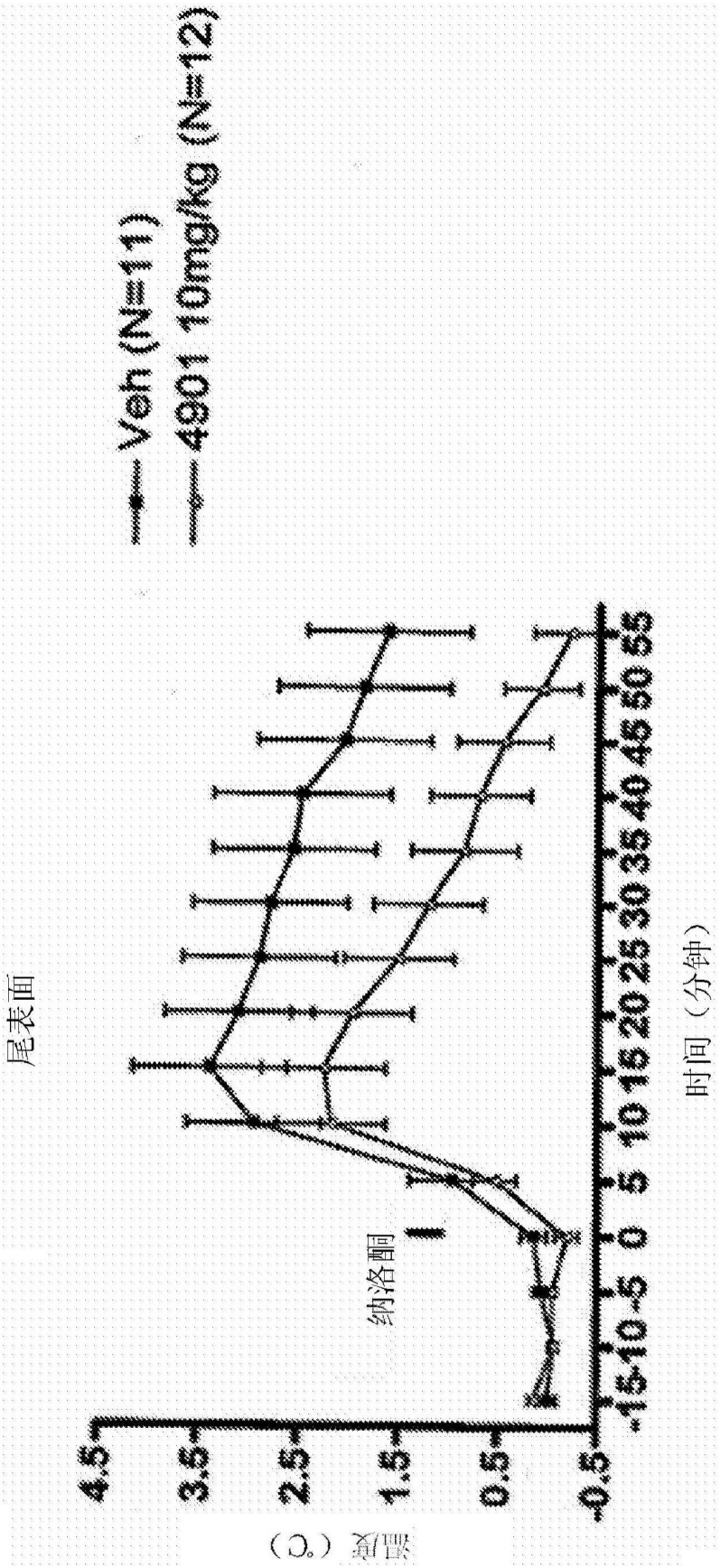

Veh (N=11)
4901 10mg/kg (N=12)
尾表面
纳洛酮
温度（°C）
时间（分钟）
4.5
3.5
2.5
1.5
0.5
-0.5
-15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55

图11B

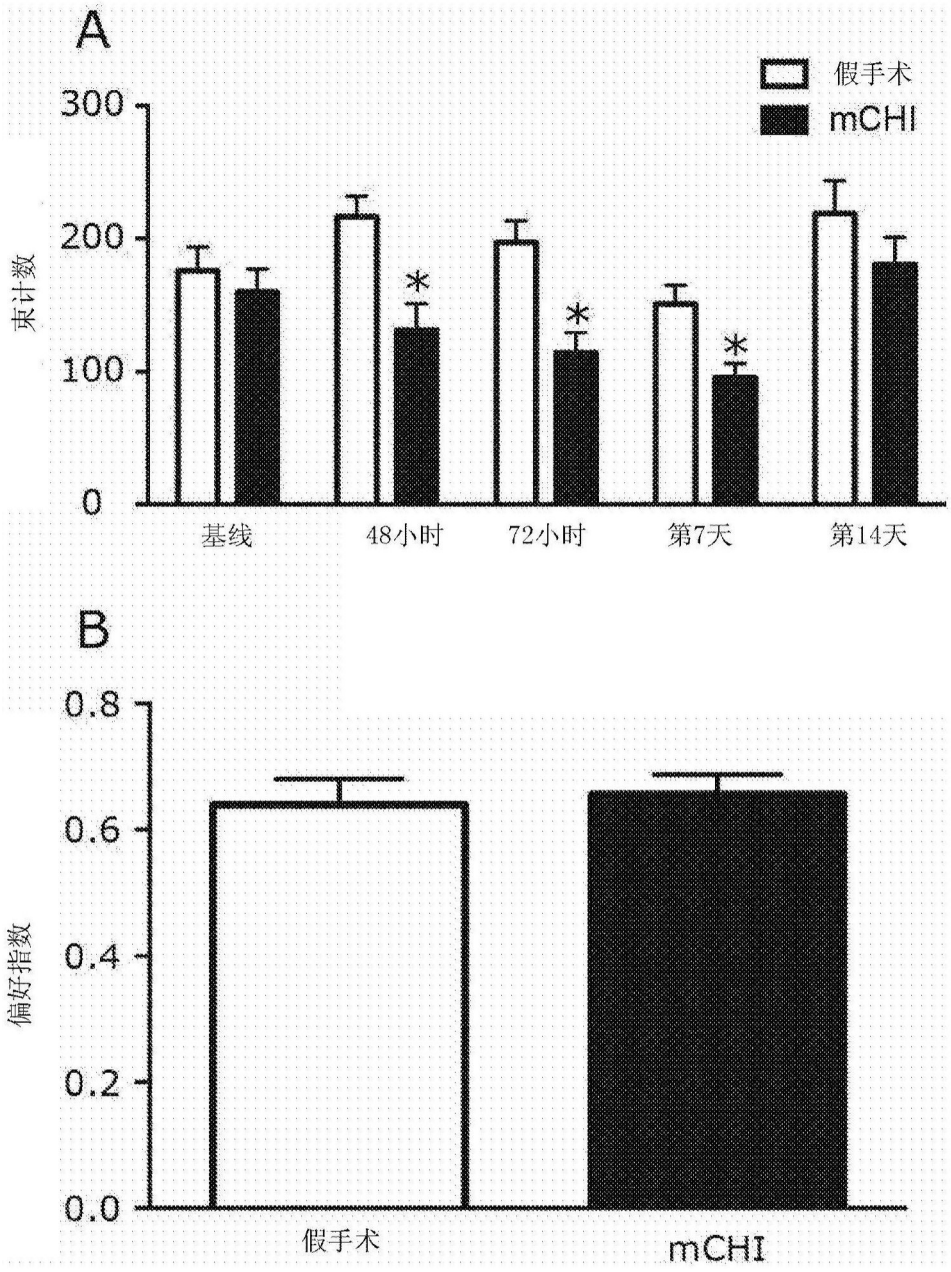

A
假手术
mCHI
300
200
100
0
束计数
*
*
*
基线
48小时
72小时
第7天
第14天
B
0.8
0.6
0.4
0.2
0.0
偏好指数
假手术
mCHI

图12A和图12B

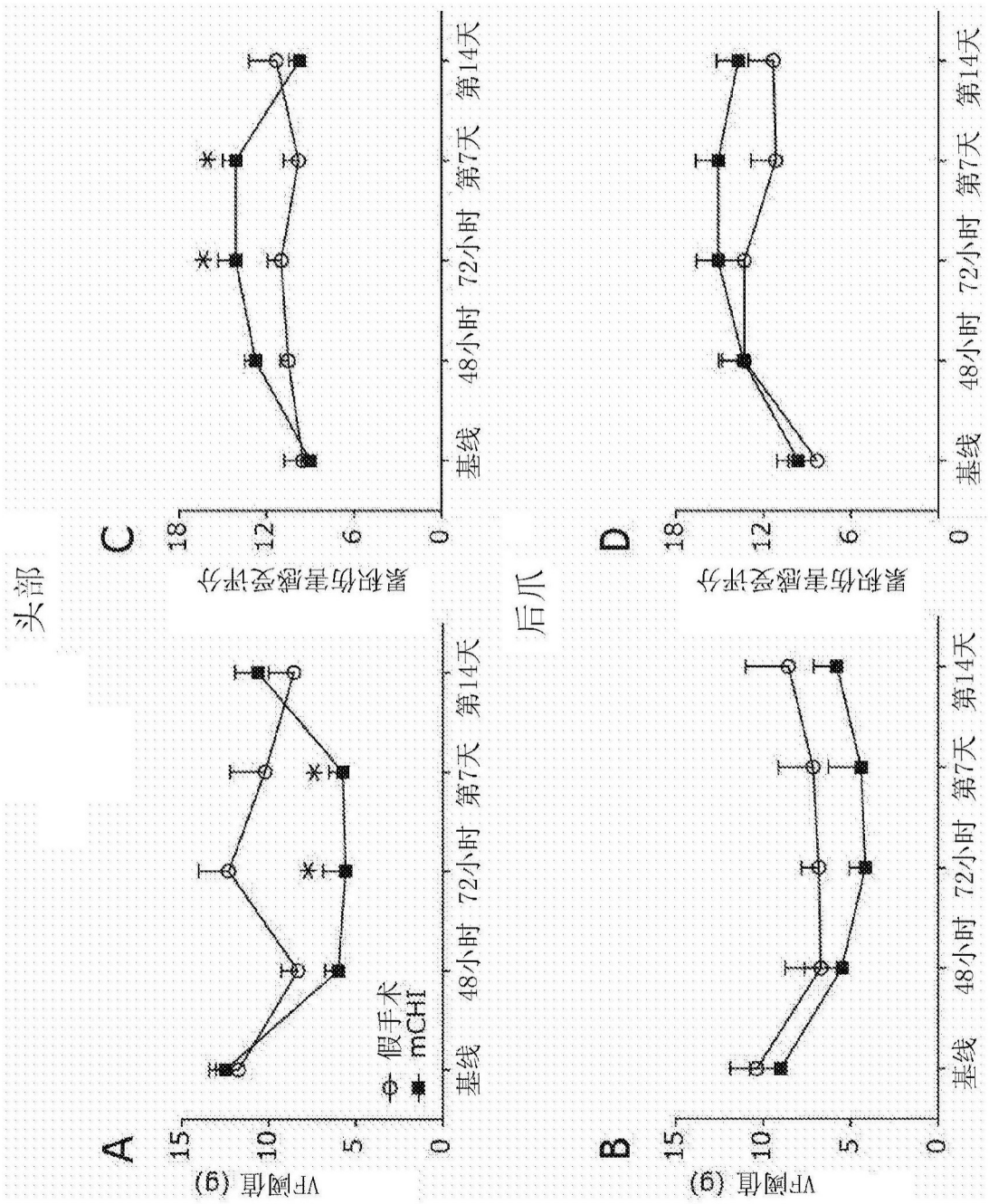

A
B
C
D
头部
后爪
VF阈值 (g)
累积伤害感受评分
假手术
mCHI
基线
48小时
72小时
第7天
第14天
0
5
10
15
6
9
12
18

图13A至图13D

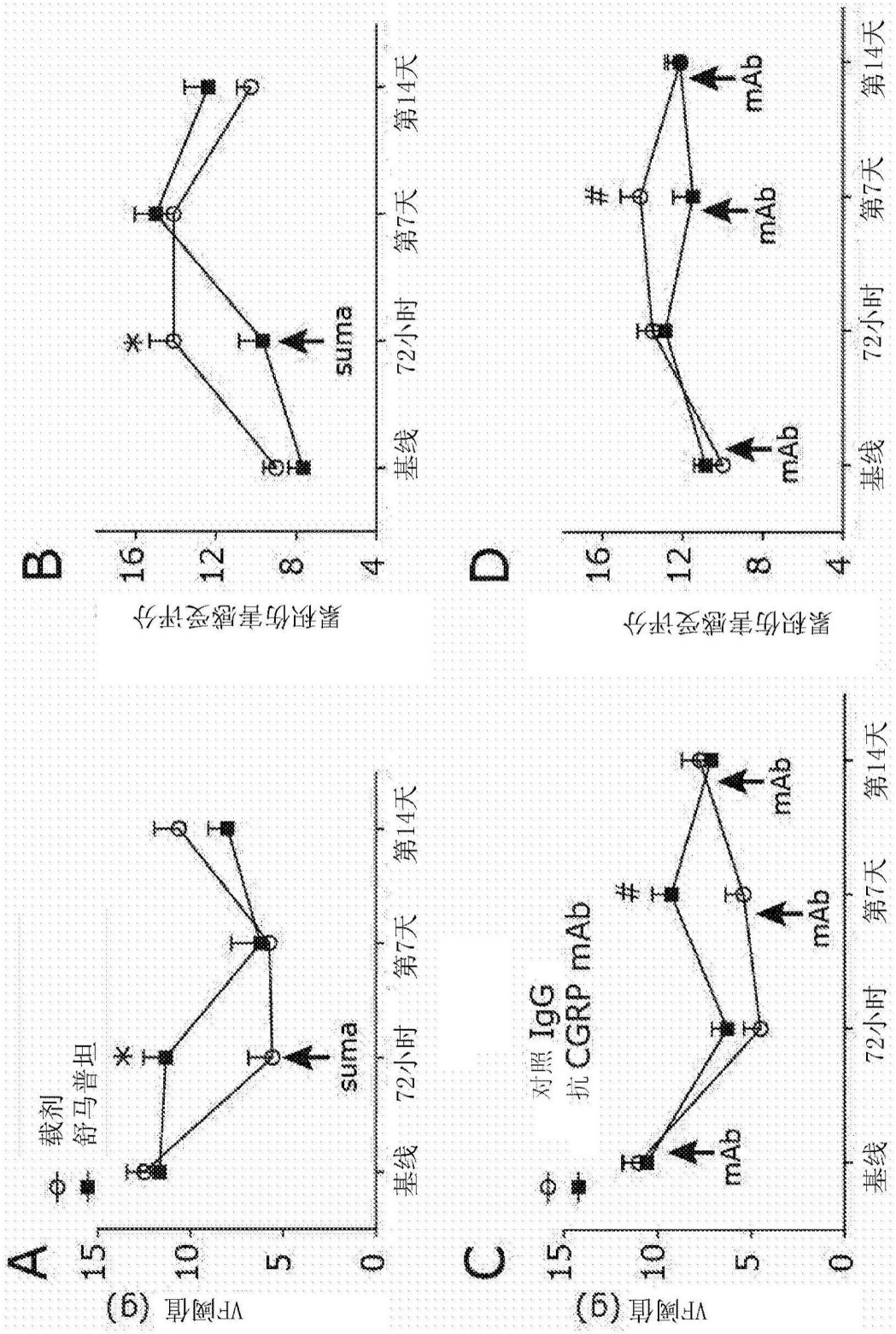

A
B
C
D
载剂
舒马普坦
对照 IgG
抗 CGRP mAb
VF阈值 (g)
累积伤害感受评分
基线
72小时
第7天
第14天
suma
mAb
#
*

图14A至14D

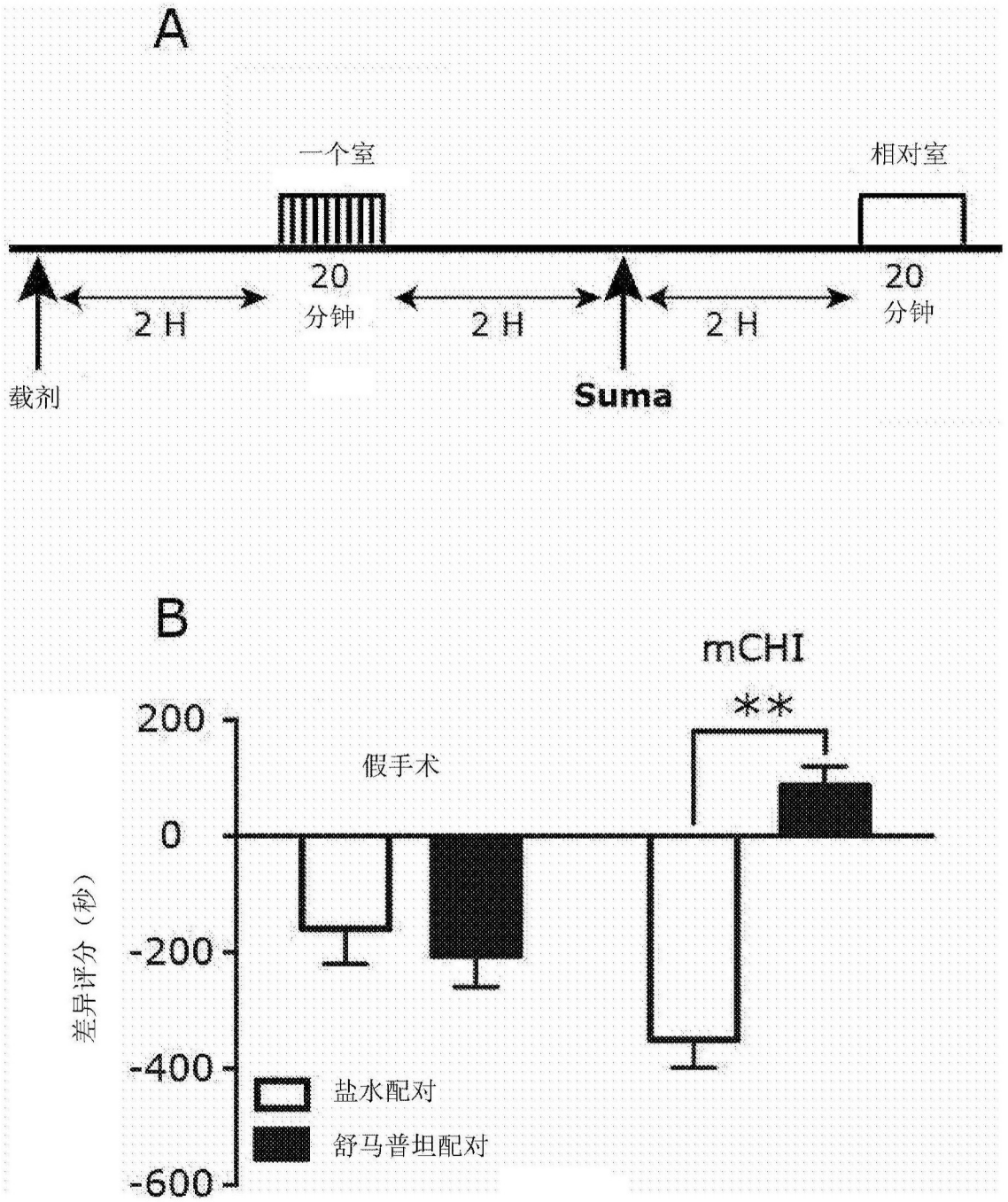

A
一个室
相对室
20
分钟
2 H
2 H
2 H
20
分钟
载剂
Suma
B
mCHI
**
200
0
-200
-400
-600
假手术
差异评分（秒）
盐水配对
舒马普坦配对

图15A和15B

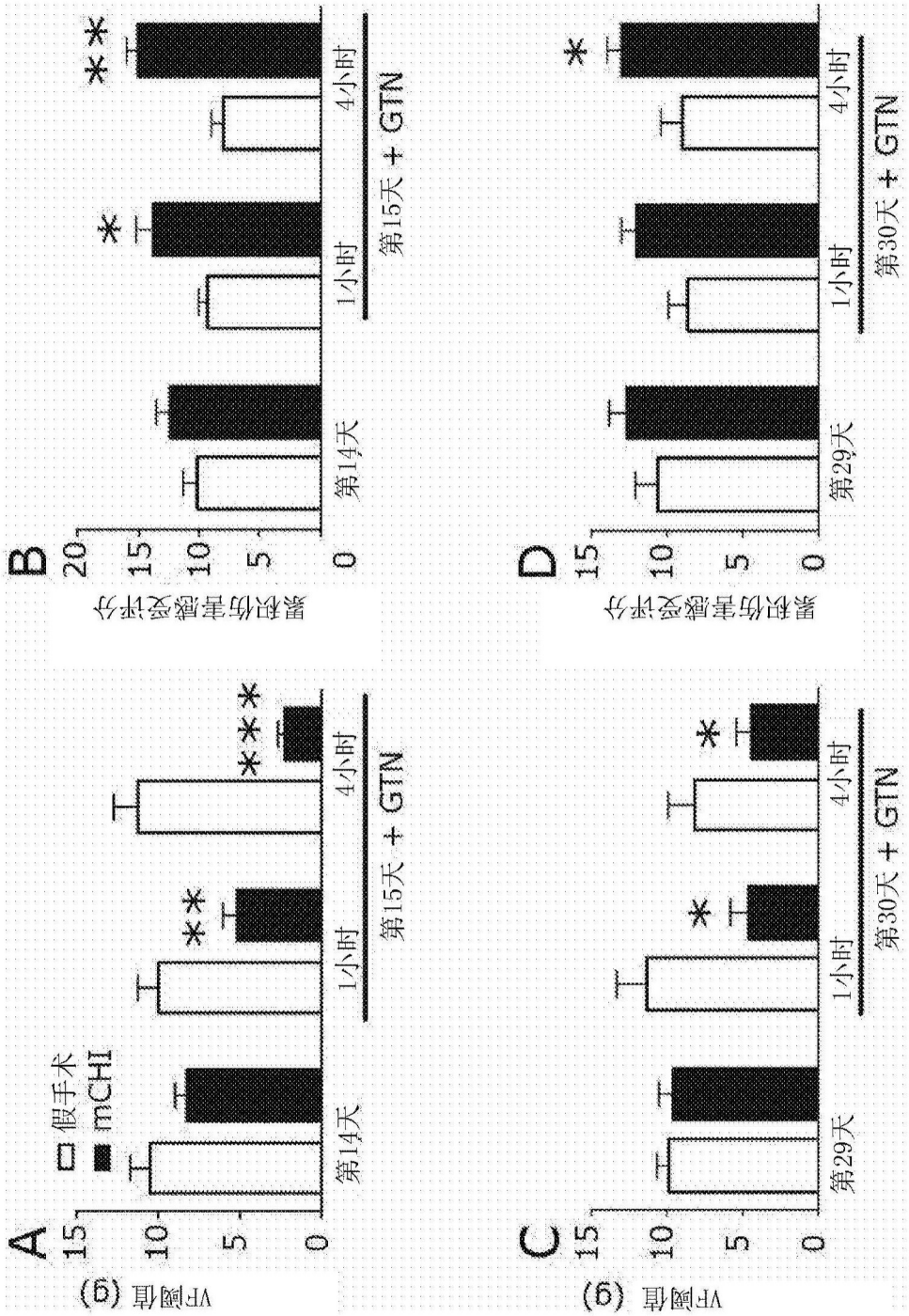

A
B
C
D
□假手术
■mCHI
VF阈值 (g)
累积伤害感受评分
第14天
第29天
第15天 + GTN
第30天 + GTN
1小时
4小时
0
5
10
15
20
*
**
***

图16A至16D

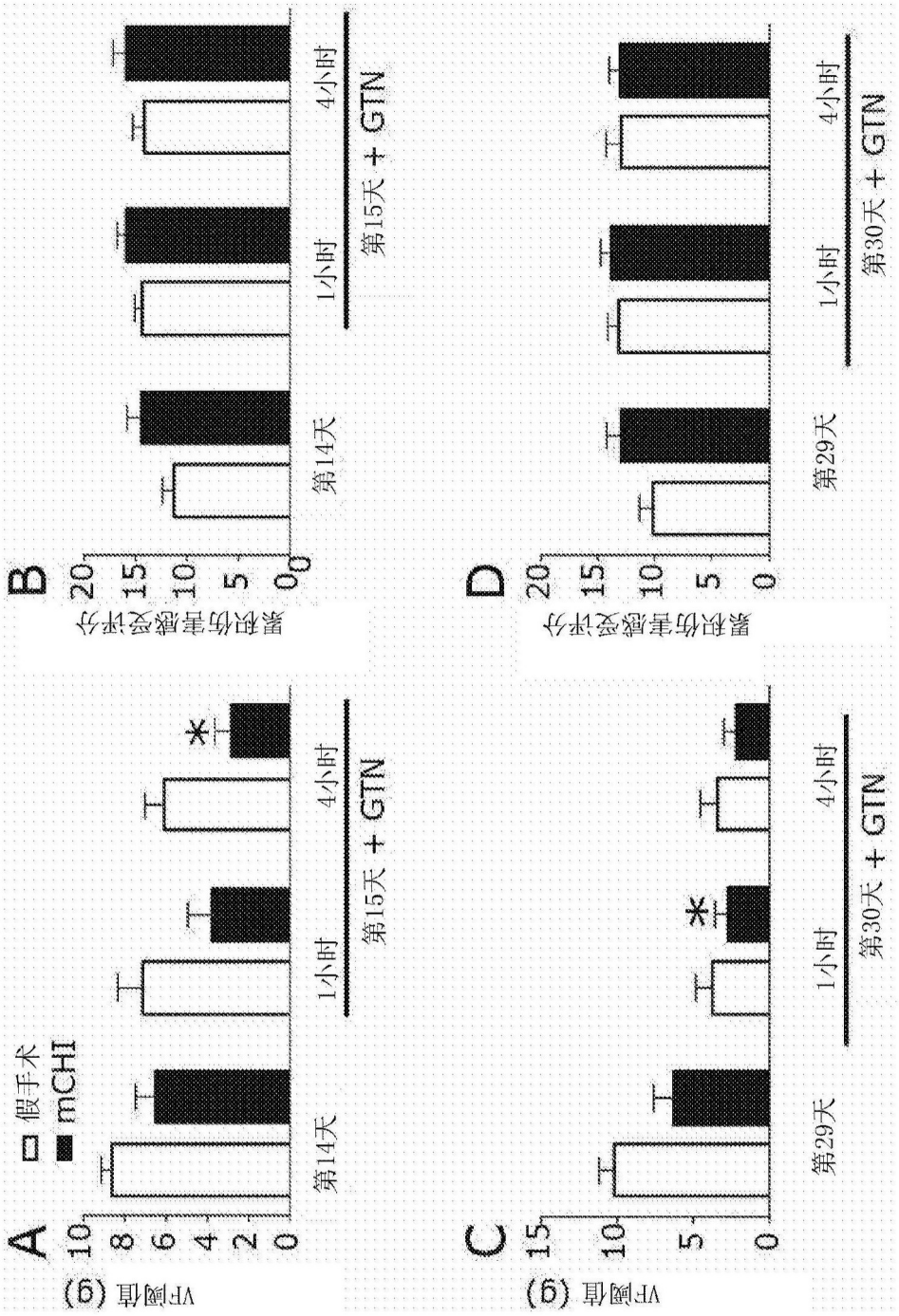

A
B
C
D
假手术
mCHI
VF阈值 (g)
累积伤害感受评分
第14天
第29天
1小时
4小时
第15天 + GTN
第30天 + GTN
*

图17A至17D

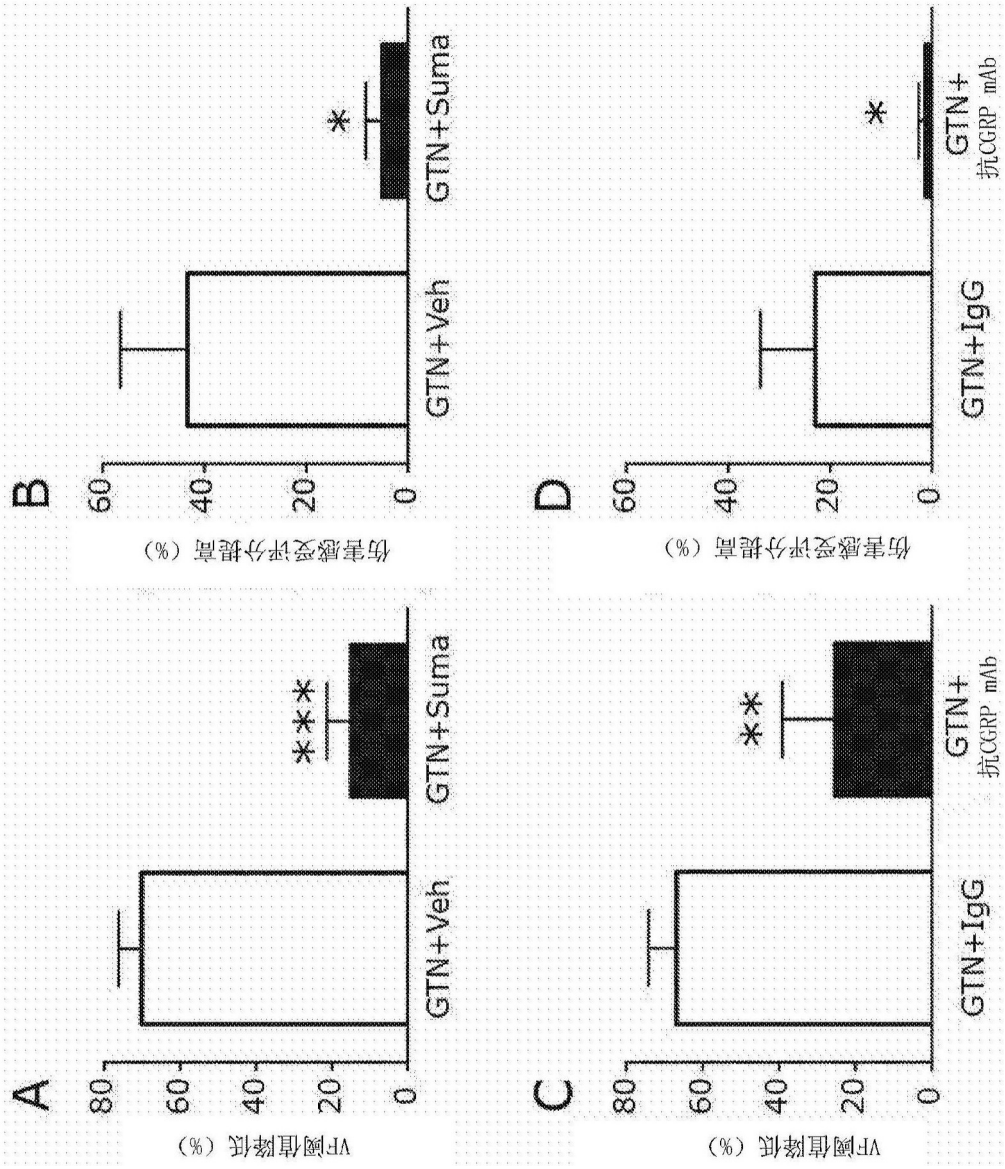

A
B
C
D
VF阈值降低（%）
伤害感受评分提高（%）
0
20
40
60
80
GTN+Veh
GTN+Suma
GTN+IgG
GTN+
抗CGRP mAb
*
**
***

图18A至18D

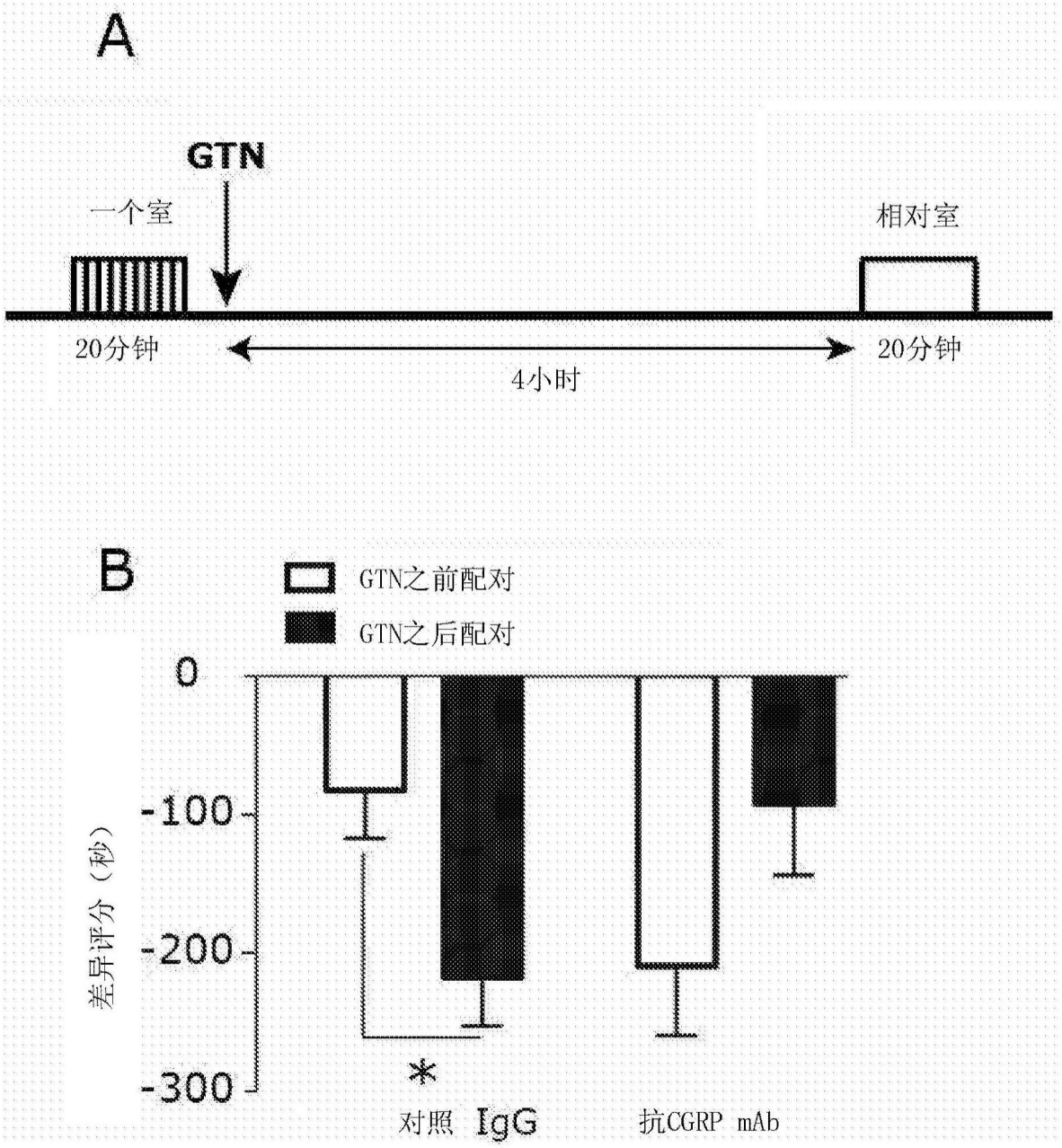

A
GTN
一个室
相对室
20分钟
4小时
20分钟
B
GTN之前配对
GTN之后配对
0
-100
-200
-300
差异评分（秒）
*
对照 IgG
抗CGRP mAb

图19A和19B