Headache
© 2013 American Headache Society

ISSN 0017-8748
doi: 10.1111/head.12179
Published by Wiley Periodicals, Inc.

Review Article

Calcitonin Gene-Related Peptide (CGRP) and Migraine Current Understanding and State of Development

Marcelo E. Bigal, MD, PhD; Sarah Walter, PhD; Alan M. Rapoport, MD

Calcitonin gene-related peptide (CGRP) is a ubiquitous neuropeptide found at the very centers of the migraine process, both centrally and peripherally. It has been under careful study for approximately 25 years. Several CGRP-receptor antagonists are being evaluated for acute treatment of episodic migraine. Three monoclonal antibodies are being studied for prevention of episodic migraine, and 1 monoclonal antibody is being studied for prevention of chronic migraine. In this review, we discuss the role of CGRP in normal physiology and the consequences of CGRP inhibition for human homeostasis. We then review the current state of development for CGRP-receptor antagonists and CGRP monoclonal antibodies. We close by speculating on the potential clinical role of CGRP antagonism in the acute and preventive treatment of episodic and chronic migraine.

Key words: calcitonin gene-related peptide, antibodies, migraine, chronic migraine

(*Headache* 2013;53:1230-1244)

Calcitonin gene-related peptide (CGRP) is a 37-amino-acid neuropeptide that is derived from the gene encoding calcitonin by alternative splicing of mRNA and proteolytic processing of its precursor.[1,2] Despite their common origin, calcitonin and CGRP are involved in totally different physiological processes in humans. While calcitonin is mainly related to calcium homeostasis and bone remodeling, CGRP is involved in vasodilation and sensory transmission.

CGRP is found in literally every organ system in the body,[3] occurring in 2 isoforms, α- and β-CGRP.[4,5] α-CGRP is the predominant form in the peripheral nervous system, while the β-isoform is mainly present in the enteric nervous system.[6] CGRP is highly conserved across species,[7] suggesting that the neuropeptide is of importance in functions that were established relatively early in mammalian evolution.

Immunohistochemistry demonstrated that CGRP is mainly produced in the cell bodies of both ventral and dorsal root neurons.[8] Radioimmunology further demonstrated that this molecule is especially common in the trigeminal system, where up to 50% of the neurons produce it.[9] Indeed, the potential role of CGRP in migraine pathophysiology was suggested more than 20 years ago,[10,11] and since then, our knowledge of the peptide and its role in the pathophysiology of migraine has increased substantially and has

From the Chief Medical Officer, Labrys Biologics, Inc, San Mateo, CA, USA (M.E. Bigal); Director of Preclinical Research, Labrys Biologics, Inc, San Mateo, CA, USA (S. Walter); Department of Neurology, The David Geffen School of Medicine at UCLA, Los Angeles, CA, USA (A.M. Rapoport).

Address all correspondence to M. E. Bigal, Labrys Biologics, Inc., 1810 Gateway Drive, Suite 230, San Mateo, CA 94404, USA, email: mbigal@labrysbiologics.com

Accepted for publication June 7, 2013.

Conflict of Interest: Drs. Bigal and Walter are employees of Labrys Biologics, Inc. San Mateo, CA, USA. Dr. Rapoport is on the speaker's bureau of Allergan, Impax, and Nautilus Neurosciences. He is a consultant to Doctor Reddy's, Impax, Merck, Nautilus Neurosciences, NuPathe, Transcept, and Winston.

led to a robust interest in targeting CGRP to treat migraine. This interest is well illustrated by a recent "year in review" paper which claims that "2012 might be remembered as the year of CGRP antagonists (despite the hurdles). At present, CGRP remains the most actively evaluated target in migraine drug research."[12]

The search for an effective CGRP antagonist has become increasingly exciting now that development is being pursued not only with receptor antagonists, but with antibodies to CGRP and its receptors.[13] In this paper, we review this subject. We start by discussing the role of CGRP in normal physiology and the consequences of CGRP inhibition for human homeostasis. We then review clinical development of CGRP inhibition for the acute treatment of migraine. We follow with a description of the current state of development of CGRP-receptor antagonists (CGRP-RA) and CGRP monoclonal antibodies (CGRP-mAb), focusing on similarities and differences in the pharmacological development of these 2 subclasses. We close by speculating on the potential clinical role of CGRP antagonism in the acute and preventive treatment of episodic and chronic migraine (CM).

THE PHYSIOLOGICAL ROLE OF CGRP

CGRP is distributed throughout the central and peripheral nervous systems and is often colocalized with other peptides in C fibers.[14] α-CGRP is the most abundant isoform and is found in several areas of the central and peripheral nervous system.[15] β-CGRP, which differs from α-CGRP by only 3 amino acids, is primarily located in the gut at the terminal endings of enteric nerves.[16]

Both isoforms of CGRP are potent natural vasodilators. CGRP exhibits a range of biological effects on tissues, including those associated with gastrointestinal, respiratory, endocrine, and central nervous systems (CNS).[17,18] CGRP may also have a role in promoting tumor-associated angiogenesis and tumor growth.[19] Nonetheless, the role of CGRP has been more extensively studied in the context of its vascular and nociceptive functions detailed in this paper.

ROLE OF CGRP IN CARDIOVASCULAR HOMEOSTASIS

CGRP is one of the most potent endogenous vasodilators, and its role in the control of blood pressure under normal and abnormal circumstances, including cardioprotection against ischemia/reperfusion injury, has received considerable attention.[20-23] If CGRP truly plays such an important vascular role, CGRP agonists could be developed for the management of hypertension and coronary syndromes, while CGRP antagonists should have their safety meticulously demonstrated.

The vascular role of CGRP has been superbly reviewed by Brain and Grant.[24] Although CGRP has overall vasodilatory properties, the microvasculature responds strongly to the molecule. At this level, its potency is around 10-fold greater than the prostaglandins and 100-1000 times greater than other classic vasodilators.[24] In addition to its potency, CGRP also differs from other vasodilator substances in that it has a particularly long duration of action. Small doses injected into human skin produce an erythema that lasts for 5-6 hours,[25] a fact that has important research implications. One of the most commonly used assays to screen for potentially effective CGRP antagonists involves applying topical capsaicin to the skin of animals (or humans). Capsaicin strongly induces the local release of CGRP which results in quantifiable vasodilation. This assay provides a platform for testing the efficacy of compounds targeting CGRP by quantifying their ability to reverse or prevent vasodilation.[26]

The vasodilatory activity of CGRP extends to a wide variety of tissues and organs, and is particularly potent in the cerebral circulation.[27] At the time CGRP was first characterized, migraine was viewed as a "vascular headache." Therefore, considerable interest was paid to the role of CGRP in migraine. An early rationale was that the release of CGRP (by activation of the trigeminal nuclei) would lead to vasodilation of the small arteries in the trigeminal distribution with vascular edema and perivascular inflammation.[27-30] Indeed, jugular levels of CGRP seem to be increased during migraine attacks, and intravenous CGRP administration induces migraine-like headache in most individuals with migraine.[31-33] As discussed later in this paper, the role of CGRP in

migraine headaches has since been shown to go far beyond its effects on the vascular compartment.

The vasodilatory activity of CGRP and its wide distribution ensure that, in addition to regulating tissue blood flow under physiological conditions, it is in a prime position to protect tissues from injury. Animal studies showed that infusion of CGRP decreased the likelihood of onset of ischemia-reperfusion arrhythmias.[34] In animal ischemia models, CGRP was found to improve the contractile function of the heart in dogs[35] and pigs.[21] However, studies failed to demonstrate that CGRP, when given during ischemia, had any protective effect, as evidenced by reduction in infarct area.[21] To reconcile these findings (CGRP seemed to improve functional outcomes after ischemia, but did not decrease infarct area), it has been speculated that CGRP has a role in preconditioning, or on the ability of tissues to endure ischemia after previous ischemic episodes.[36]

Many of the theoretical concerns that emerged from the in vitro and in vivo characterization of CGRP and its receptors were investigated in human studies during the robust development of the CGRP-RAs. Relevant findings are summarized later.

WHAT ARE THE CARDIOVASCULAR CONSEQUENCES OF INHIBITING CGRP IN HUMANS?

Based on the physiological role of CGRP, 4 major cardiovascular effects could be of concern with CGRP inhibition: medication-induced hypertension, counterbalancing the effect of antihypertensive drugs that have vasodilatory properties, inhibition of stress (or ischemia)-induced vasodilation, and impairment of cardioprotective mechanisms (Table 1).

The Risk of Inducing Vasconstriction.—Although CGRP is a potent vasodilator, in vitro and in vivo studies repeatedly showed that CGRP antagonists (receptor antagonists and antibodies) do not have vasoconstrictor activity. An in vitro study showed that telcagepant, a CGRP-RA, does not cause vasoconstriction of the coronary arteries, in contrast to what was seen with 5-$HT_{1B/D}$ receptor agonists.[37] Similarly, different CGRP antagonists showed no effect on the coronaries of dogs under ischemic conditions, while 5-$HT_{1B/D}$ receptor agonists worsened the infarct area.[38]

Table 1.—Theoretical Risks of Inhibiting CGRP and Preclinical or Clinical Findings With CGRP Antagonists

Potential Risk of Inhibiting CGRP	Findings With CGRP Antagonists
Inhibition of CGRP could cause vasoconstriction mainly in the small arteries	• In vitro, telcagepant did not cause vasoconstriction of the coronary arteries, while 5-$HT_{1B/D}$ receptor agonists did.[37] • In dogs, different CGRP antagonists had no effect on the coronaries under ischemic conditions, while 5-$HT_{1B/D}$ receptor agonists had.[38] • In humans, olcegepant had no effect on global or regional cerebral blood flow, or on the blood flow velocity in the middle cerebral artery. • CGRP antagonists seem to restore normal tonus in already dilated arteries, but do not cause abnormal constriction.[40]
Inhibition of CGRP could counterbalance the clinical effect of antihypertensive drugs that have vasodilatory mechanisms	• A placebo-controlled, double-blind study tested the effects of telcagepant given after nitroglycerin and no vasoconstrictor effect of telcagepant could be demonstrated.[41]
CGRP antagonists could inhibit compensatory vasodilation during ischemia.	• Patients with exercise-induced stable angina received supratherapeutic doses of telcagepant or placebo during treadmill exercise time (TET) and no significant differences were seen between groups, including time to angina.[42]

CGRP = calcitonin gene-related peptide.

The first CGRP-RA to be tested in humans, olcegepant, was given to healthy volunteers in a double-blind, placebo-controlled, crossover study. Transcranial Doppler was used to measure the middle cerebral artery blood flow velocity, and photon emission computed tomography measured global and regional cerebral blood flow. Absolutely no effects on systemic hemodynamics were observed.[39] Interestingly enough, studies suggest that this and other CGRP antagonists restore normal tonus in already dilated arteries but do not cause abnormal constriction.[40]

The Risk of Inhibiting Vasodilatory Medications.— To test whether CGRP antagonism could affect the vasodilation induced by certain antihypertensive medications, 500 mg telcagepant or placebo were given to healthy volunteers, followed 1.5 hour later by 0.4 mg nitroglycerin (NTG). Blood pressure, aortic augmentation index (AIx), and brachial artery diameter (BAD) were measured. The aortic AIx following NTG decreased by –18.5% after telcagepant vs –17.3% after placebo. The BAD fold increase following NTG was 1.14 after telcagepant vs 1.13 after placebo. No vasoconstrictor effect of telcagepant could be demonstrated.[41]

The Risk of Inhibiting Vasodilation During Ischemia.—Considering the role of CGRP in vasoresponse during ischemia, one might hypothesize that CGRP-receptor antagonism could reduce coronary vasodilatory capacity. To explore this topic, the effects of supratherapeutic doses of telcagepant (600 or 900 mg) on treadmill exercise time (TET) were assessed in a double-blind, placebo-controlled study in patients with reproducible exercise-induced stable angina with ischemic ST-segment depression. Patients performed treadmill exercise at T_{max} (2.5 hours after the dose). The incidence of ischemic ST-segment depression ≥1 mm was 83.9% in those receiving placebo, 90.7% in those receiving telcagepant 600 mg, and 85.7% in those receiving telcagepant 900 mg. TET was not significantly different across groups, and all other data were similar across groups. The authors suggested that the broad redundancy in vasodilatory mechanisms might preserve the compensatory vasodilatory response during myocardial ischemia, even in the presence of CGRP-receptor antagonism.[42]

The available data are insufficient to rule out all cardiovascular safety concerns with inhibiting CGRP function. But no other class of migraine medication, including those inducing vasoconstriction such as ergotamine and the triptans,[43-45] has been so intensively and exhaustively tested in this regard.

ROLE OF CGRP IN NOCICEPTION AND NEURONAL PLASTICITY

Although the original function of CGRP was likely related to maintaining vascular homeostasis, it has been speculated that CGRP largely lost its vascular functions during evolution and should now be seen as a neuropeptide with an important function in nociceptive transmission.[46,47] For a review of the role of the role of CGRP on other neurological functions, the reader is referred to.[48]

As mentioned, CGRP is widely expressed in the central and peripheral nervous systems where it appears to modulate the function of other neurotransmitters.[49,50] In the trigeminal ganglion, it is often coexpressed with substance P and $5\text{-}HT_{1B/D}$ receptors.[51-53] The satellite glial cells of the trigeminal ganglion also express CGRP receptors.[54] These cells seem to have a pivotal role in modulating neuronal metabolism via gap junctions.[55]

The clinical correlation of these very peripheral actions of CGRP has to do with the neurovascular inflammation that seems to be of importance for migraine.[3,56] The release of CGRP at trigeminal nerve endings induces vasodilation (and edema) and dural mast cell degranulation, which both contribute to neurogenic inflammation, a sterile form of inflammation secondary to sensory nerve activation.[57] Furthermore, as stated by Raddant and Russo,[3] "The inflammatory cascade can be triggered by CGRP actions on dural mast cells and satellite glial cells of the trigeminal ganglion."

The peripheral CGRP-containing neurons (in the trigeminal ganglion and elsewhere) are polymodal nociceptors that innervate essentially all peripheral tissues and send primary afferent input to the dorsal horn, trigeminal nucleus caudalis, or nucleus of the solitary tract (which, in turn, project to the brainstem, amygdala, hypothalamus, and thalamic nuclei).[48] CGRP-containing neurons in the trigeminal ganglion project to the trigeminal nucleus caudalis and C1-C2, where CGRP also acts post-junctionally on these second-order neurons to transmit pain signals from the brainstem to the thalamus.[58,59]

The clinical correlation of CGRP actions at the level of the trigeminal nucleus caudalis is relevant as well. The brainstem has a key role in the pathophysiology of migraine.[60,61] Brainstem stimulation causes activation of the trigeminovascular system, resulting in peripheral CGRP release and neurogenic inflammation (described earlier).[62,63] Furthermore, activation of the brainstem is associated with altered

perception termed allodynia (a condition in which nonpainful stimulation is perceived as painful) as well as with the development of second- and third-order neuronal sensitization.[64,65] Accordingly, if we understand migraine as the combined result of altered perception of stimuli that are usually not painful, as well as the activation of a feed-forward neurovascular dilator mechanism in the first (ophthalmic) division of the trigeminal nerve, we realize that CGRP is involved in the pathophysiology of migraine both centrally and peripherally.[66]

CGRP and its receptors are widely distributed across other parts of the CNS as well, in areas that are relevant to pain and in areas that may not be, such as the cerebellum.[67,68] The function of CGRP in these areas is not well understood. Studies have suggested that CGRP is expressed in areas that could explain migraine-related photophobia.[69] In a model of transgenic mice, light-aversive behavior was greatly enhanced by intracerebroventricular injection of CGRP and blocked by coadministration of the CGRP-RA olcegepant.[70]

Finally, CGRP seems to be important in determining neuronal plasticity and synapse formation. This is either due to its direct actions on neurons or its indirect actions on the glia via its modulatory actions.[71-73]

In summary, CGRP and its receptors are largely expressed in neurons and glia, both peripherally and centrally. As discussed later, this broad expression has relevance for drug development. Pain improvement can be achieved by blocking CGRP peripherally, centrally, or both, and brain penetration may not be essential for the analgesic properties of CGRP antagonists. Brain penetration could have direct influence on photophobia and other neurological symptoms of migraine, which would be of importance for the acute treatment of migraine but not necessarily for the preventive treatment of migraine. The flip side of central penetration would be disturbing the homeostatic role of CGRP at the neurons, including its actions on neuroplasticity. It is of interest that CGRP is largely expressed in the cerebellum, which only recently has been implicated as modulating nociceptive processing,[74] and which seems to be a controversial target area for migraine complications such as stroke.[75,76] Sporadic administration of brain-penetrating CGRP antagonists for the acute treatment of migraine would likely not affect this homeostasis, but chronic administration with the goal of providing preventive treatment would have to have its safety demonstrated in animal models.

CLINICAL DEVELOPMENT – TARGETING CGRP

CGRP can be targeted in several ways. The best explored mechanism is to antagonize CGRP receptors using small molecules (CGRP-RA) that compete with CGRP for a binding pocket or cleft produced by RAMP1 and the CGRP receptor. Free CGRP and CGRP receptors can also be targeted using monoclonal antibodies (mAbs) that can bind and neutralize biological activity.[13]

Four distinct CGRP-RA (the "gepants") have demonstrated proof of efficacy, but all were discontinued for a variety of reasons. They are summarized in Table 2 and described later.

Olcegepant (BIBN4096BS) was the first CGRP antagonist to be developed. Dose-responsive clinical efficacy was achieved. Intravenous doses ranged from 0.25 to 10 mg, and the 2.5 mg dose was considered to be ideal with a response rate of 66%, as compared with 27% for placebo ($P = .001$). Pooled together, all doses had a response rate of 60%. Onset of effect occurred 30 minutes post dose. Adverse events happened in 20% vs 12% in those receiving placebo.[77] Olcegepant was discontinued because of difficulties in developing an oral formulation.

Telcagepant (MK-0974) was the first orally available CGRP-RA. In the Phase 2 clinical trial, an adaptive design was used to test doses ranging from 25 to 600 mg against 10 mg rizatriptan and placebo. Doses of 300 mg, 400 mg, and 600 mg were given. Pain relief proportions at 2 hours were 68.1% (300 mg), 48.2% (400 mg), and 67.5% (600 mg) relative to 69.5% (rizatriptan) and 46.3% (placebo). Tolerability was excellent, better than rizatriptan and comparable to placebo.[78]

Based on the results of Phase 2, doses of 150 mg and 300 mg telcagepant were carried to Phase 3. The first pivotal study used 5 mg zolmitriptan as the active

Table 2.—Summary of Clinical Data Obtained in Phase 2 and 3 Studies on the CGRP Receptor Antagonists (the "Gepants")

	2 Hours Pain Relief (%)	2 Hours Pain-Free (%)	Adverse Events (%)†
Olcegepant (phase 2)[77]			
2.5 mg	66	44	25
Placebo	27	2	12
Telcagepant			
Study 1 (phase 2)[78]	68.1	45.2	35.3
300 mg	69.5	33.4	42
Rizatriptan 10 mg	46.3	14.3	36.2
Placebo	21.8	30.9	–0.7
Drug – Placebo			
Study 2 (first pivotal)[79]			
150 mg	50.2	17.2	31.4
300 mg	55.4	26.9	37.2
Zolmitriptan 5	56.1	30.8	50.7
Placebo	26.8	9.4	32.1
Study 3 (Second pivotal)[115]			
150 mg	53.8	22.6	30.7
300 mg	56	23.6	34.6
Placebo	32.7	10.4	30.9
MK 3207 (phase 2)[82]			
100 mg	52.5	23.7	30.6
200 mg	69	36.2	27
Placebo	36.1	9.8	20.4
BI44370A (phase 2)[84]			
200 mg‡	50.8	21.5	6.2
400 mg	56.2	27.4	9.6
Eletriptan 40 mg	56.5	34.8	17.4
Placebo	18.6	8.6	10

†Methods to assess AEs varied from trial to trial, so cross-study comparisons should not be performed.
‡Nonsignificant for the primary endpoint (2-hour pain-free).
CGRP = calcitonin gene-related peptide.

comparator and was the largest clinical study of a CGRP-RA conducted to date, with 1380 patients being randomized. Telcagepant (300 mg) had similar 2-hour efficacy to zolmitriptan (5 mg); both were superior to 150 mg telcagepant, which was superior to placebo. Tolerability was similar to placebo: adverse events were recorded for 31% taking telcagepant 150 mg, 37% taking telcagepant 300 mg, 51% taking zolmitriptan 5 mg, and 32% taking placebo.[79] However, when the pooled results of telcagepant were analyzed, the data suggested that telcagepant had a slow onset but a long duration of action; overall efficacy at early time points was low relative to triptans but the recurrence of pain was also reduced compared with triptans.[13] In spite of the promising clinical efficacy data, telcagepant development was discontinued because of concerns regarding liver toxicity. Elevations of hepatic enzymes were seen in some participants in a Phase IIa study where telcagepant was given twice daily for the prevention of migraine. Similar elevations were seen in a short-term study of menstrual migraine.[13,80]

A third CGRP-RA, MK-3207, was 40- to 65-fold more potent than telcagepant[81] and was tested in an adaptive design exploring doses from 2.5 to 200 mg. The 100 and 200 mg doses yielded pain-free rates of 23.7% and 36.2% (placebo = 9.8%), and pain relief rates of 52.5% and 69% (placebo = 36.1%).[82] Similar to other compounds in the same class, tolerability was excellent but development was also discontinued because of concerns related to liver toxicity.[83]

Finally, a Phase 2 trial tested BI44370A in 341 patients. Doses ranged from 50 to 400 mg, and were compared with placebo and 40 mg eletriptan. The primary endpoint, 2-hour pain freedom, was achieved significantly more often by patients receiving the 400 mg dose (27.4%) and eletriptan (34.8%) than placebo (8.6%). Other doses were not significantly different from placebo for the primary endpoint. Tolerability was excellent.[84]

In addition to demonstrating proof of efficacy, the CGRP-RA clinical trials also demonstrated the extraordinary tolerability of this class. The issue was best explored in the development of telcagepant, where in addition to the large pivotal studies, a distinct clinical trial was conducted specifically to evaluate its long-term tolerability for acute treatment of migraine attacks. The trial consisted of 1068 patients. A total of 19,820 attacks were treated with telcagepant (mean per patient = 31) and 10,981 with rizatriptan (mean per patient = 35). Both regimens were well tolerated but fewer drug-related adverse events (difference: –15.6%; 95% CI –22.2, –9.0) were reported for telcagepant vs rizatriptan.[85]

Other CGRP-RAs are being developed and, at the time of this writing, clinicaltrial.gov lists 2 of them: BMS-927711 is listed in Phase 1,[86] and MK-1622 is in Phase 2B, with doses ranging from 1 to 100 mg, for the acute treatment of migraine attacks.[87]

mAbs

mAbs, or antibodies produced by a single clone of cells, were first shown to have therapeutic activity in 1982, when a patient with lymphoma experienced a complete response when given antibodies against his tumor cells produced in mice.[88] In the past 20 years, their clinical utility has expanded dramatically with more than 20 mAbs that are Food and Drug Administration (FDA)-approved for human use.[89]

mAbs from nonhuman species (eg, mice) may generate strong immunological reactions when given to humans. Although this may not be a problem for short-term interventions, it becomes a major hurdle for chronic use. As a first attempt to reduce immunogenicity, chimeric antibodies were engineered where murine constant AB regions were replaced by human constant regions.[90] The next development was the humanization process which resulted in antibodies where only the complementarity determining regions of the variable regions are of mouse-sequence origin. Fully human antibodies use human amino acid sequence-derived antibody regions where antigen specificity has been selected either in vivo by the use of genetically modified mice or by antibody engineering.[91] Fully human and humanized antibodies carry a lower risk for inducing immune responses in humans than mouse or chimeric antibodies.[92]

PRECLINICAL DEVELOPMENT STRATEGIES FOR CGRP-RA AND mAb – DIFFERENCES AND CHALLENGES

Preclinical studies to support clinical testing are critical to the development plan for any new therapeutic, whether it be a traditional small molecule or a mAb. While there are many commonalities between the studies required to support these 2 types of medications, such as pharmacokinetic (PK) assessments and repeat dose toxicology studies, there are unique challenges that come with demonstrating safety.

Antibodies are large glycoproteins produced by B-cells. They are composed of 2 heavy chains and 2 light chains held together by disulfide bonds to form a Y-shaped protein. Within each chain are conserved and variable regions; the variable region is part of the antigen recognition site and is the portion of the complex that confers antigen specificity. The utility of mAbs as therapeutic is in part due to this amazing specificity as well as their extended PK profile in humans.[93] mAbs typically have a much longer terminal half-life than small molecules which makes them especially well suited for chronic indications or preventive treatments and less useful for acute, or one-time treatments for which small molecules are better suited.

One of the first steps in preclinical testing of mAbs is species selection for in vivo safety studies. With small molecules, a rodent (rat or mouse) and a nonrodent (eg, dog) species are commonly used.[94] For mAbs, differences in epitope recognition across species may translate into differences in pharmacologic activity between preclinical species, causing toxicologists to often include nonhuman primates in their studies.

Small molecules and their metabolic subproducts can have a variety of undesirable on- and off-target effects; this is uncommon for mAbs, as their dose-limiting toxicities tend to be due to receptor-mediated interactions resulting in an exaggerated pharmacologic response.[95] Because small molecules are metabolized through reactions that can be saturated, accumulation can occur which may help define the maximally tolerated dose (MTD). For mAbs, which are cleared through protein degradation, the MTD is often not as easily defined.

Antibodies, but typically not small molecules, may induce the development of drug neutralizing and/or clearance antibodies that can result in changes to its pharmacology. Neutralizing antibodies can bind to mAbs and interfere with their function, thereby reducing their effective concentration. Clearance-enhancing antibodies can yield PK curves that drop off sharply. Sufficient exposure to support clinical dosing is a key component of any in vivo toxicity study. The appearance of clearing or neutralizing antibodies in a toxicity study can end up reducing the utility of the study.[96]

The propensity of proteins such as mAbs to induce an immunogenic response underlies the need for early development of positive control antibodies to support the required antidrug antibody assays. As clinical development proceeds, neutralizing antibody assays are often required to help characterize the nature of any immune response that is detected, as well as its biological significance.[94]

Table 3.—Preclinical Studies as a Function of Type of Drug Development

Study / Assay	Small Molecule	mAb	Comments
Immunogenicity (ADA, NAb assay)	No	Yes	Not required for small molecules although cases arise where needed
Drug-drug interaction	Yes	No	Only warranted for mAbs when MOA would suggest concern
hERG assessment	Yes	No	Cardiovascular safety to be assessed in vivo studies for mAbs
Tissue cross reactivity	No	Yes	Typically done early in mAb development to aid species selection
Metabolism	Yes	No	
Determining MTD	Yes	Yes	Can be challenging for mAbs
Genetoxicity	Yes	No	
Carcinogenicity studies	Yes	No	Not generally needed for mAbs unless MOA would suggest concern

ADA = anti-drug antibody; mAb = monoclonal antibody; MOA = mechanism of action; MTD = maximally tolerated dose; NAb = neutralizing antibody.

On the other hand, several important concerns for small molecules are less relevant for mAbs. Different from mAbs, small molecules undergo hepatic or renal metabolism, forming metabolites of unknown pharmacology or toxicity. Development needs to characterize their safety in terms of liver or renal toxicity as well as potential drug–drug interactions. Small molecules are also more likely to penetrate the brain than mAbs, and therefore, neurotoxicity studies are important. Given their mechanism of action, the concern about nonspecific drug–drug interactions is usually minimal for mAbs, as is the requirement for formal metabolism and excretion studies.[97] Biologics are presumed to be subject to normal catabolic processes that reduce them to small peptides or constituent amino acids. In addition, there is an expectation that mAbs will not penetrate cells, eliminating the need for formal genotoxicity studies.[97] Likewise, standard in vitro cardiovascular studies are often not required for mAbs. Instead, in vivo cardiovascular safety assessments as part of either safety pharmacology or chronic toxicity studies in a relevant species are deemed more appropriate.[98]

Table 3 summarizes important differences in the preclinical development of small molecules and mAbs.

mAbs ANTI-CGRP

At the time of this writing, 4 mAbs are being actively developed for the preventive treatment of episodic or CM. LY2951742 is a mAb anti-CGRP that was licensed from Eli Lilly to Arteaus Therapeutics. A Phase 1 dose-escalating study tested single intravenous (IV) doses ranging from 1 to 600 mg, as well as 150 mg given subcutaneously (SC) every other week for 6 weeks (4 doses).[99] A Phase 2a study is ongoing; testing LY2951742 administered SC once every other week for 12 weeks against placebo, for the preventive treatment of frequent episodic migraine attacks.[100]

A second antibody targeting CGRP (ALD403) is being developed by Alder Biopharmaceuticals. The safety, PKs, and efficacy of ALD403 in the prevention of frequent episodic migraine is being tested in a 24-week Phase 1b study. According to the data posted at clinicaltrials.gov, individuals with migraine are receiving a single IV injection of active drug (dose undisclosed) or placebo and are being followed for 6 months.[101]

Amgen is developing AMG 334 for the prevention of episodic migraine. Unlike the other antibodies discussed, AMG 334 targets the CGRP receptor, not the free molecule.[102] Two ongoing Phase 1b studies are testing the safety and PK profile of single and multiple ascending doses in healthy volunteers and in individuals with migraine;[103,104] the company announced plans for Phase 2 studies in the current year.

LBR-101 (formerly known as RN-307 or PF-04427429) was acquired by Labrys Biologics, Inc. from Pfizer. It is a fully humanized mAb that potently and selectively blocks the binding of human CGRP to

its receptor. LBR-101, unlike the other CGRP antibodies, is being developed specifically for the preventive treatment of CM. In Phase 1, doses ranged from 0.2 mg up to 2000 mg; a MTD has not been identified.[105] Preparations are underway to initiate a Phase 2b trial to investigate the safety and efficacy of LBR-101 in patients suffering from CM. Because it has a terminal half-life of 44-48 days, it offers the possibility of monthly dose intervals. Safety concerns have not emerged and tolerability appears to be acceptable across several doses (Bigal et al, submitted).

CLINICAL IMPLICATIONS OF FUTURE CGRP-RELATED MEDICATIONS

It is quite possible that 1 or more oral CGRP antagonists and 1 or more mAbs to CGRP will be available for the treatment of migraine. It seems that the CGRP-RAs are being positioned for the acute treatment of migraine, while mAbs are being developed for the preventive treatment of episodic or CM.

Headache specialists usually prefer to treat acute attacks of migraine with a migraine-specific medication with the dose and route of administration that has a great likelihood of success for that particular patient. Triptans are currently the preferred class of medication prescribed for this aim.[106,107] They are effective medications, available in many dosage forms and many are now generic; but, among patients receiving triptans, upwards of 40% do not have optimal responses and 20-30% of them develop a recurrent migraine attack requiring either re-dosing or a rescue medication.[108] Patients with an incomplete response to acute medications are more likely to require an increased amount of analgesics medication, resulting in a greater chance of medication overuse headache.[109] An obvious potential use of CGRP-RA is, therefore, to provide effective alternatives for the acute treatment of migraine. These medications may also be helpful for patients who have weeks with 4 headache days, as triptans should be limited to 2 days of use per week, assuming they will not induce medication overuse headache when used intermittently.

Some patients respond well to triptans, but experience 1 or more "triptan" adverse events, such as chest and neck discomfort, drowsiness, dizziness, paresthesias, among others. These adverse events (AEs) may even prevent patients from using triptans. These patients would benefit from the good tolerability of CGRP-RA.[79]

A final group of patients that could benefit from CGRP-RA are those who cannot take triptans or other vasoconstrictive medications because of their cardiovascular effects. Even when contraindications to vasoconstrictive agents do not exist, it is well established that the presence of cardiovascular risk factors negatively affects the prescription of triptans.[110] These patients could find benefit with less risk when using medications that seem to not be associated with vasoconstriction.

It is estimated that about 35% of episodic migraineurs should be offered migraine preventive therapies.[111] Currently, there are 4 FDA approved migraine preventive medications available in the United States, and many more with class A evidence for off-label use. However, a sizeable proportion of patients qualifying for prevention does not receive it and continues to have frequent attacks each month. Among those treated, some experience significant adverse events precluding their use and some receive no benefit.[112] The CGRP mAbs that are being developed for the preventive treatment of episodic migraine could certainly add value if, as expected, they can be administered infrequently and produce few adverse events.

CM is less prevalent than episodic migraine, but because of the frequency of their headaches and high degree of disability, all sufferers qualify for preventive therapy. Currently, only onabotulinumtoxinA has been approved for CM prevention.[113] Accordingly, there is an obvious need for approval of additional preventive treatment options for CM. Some off-label medications are often tried for CM, but they carry the same liabilities as when they are used for episodic migraine (need for daily use and adverse events).[114] Only one of the aforementioned mAbs is being studied for safety and efficacy in CM. The mAb being developed for the prevention of CM (LBR-101) has a long half-life and excellent tolerability. If effective, it would certainly be a convenient option for the treatment of CM.

CONCLUSION

CGRP is a well-studied, ubiquitous neuropeptide found both centrally and peripherally at the very centers of the migraine process. Several CGRP antagonists are being evaluated for acute treatment of episodic migraine and at least 4 mAbs are being studied for migraine prevention, 1 for prevention of CM. It is just a matter of time until CGRP-RAs are approved for the acute treatment of migraine given that proof of efficacy has already been established. As for the mAbs, once efficacy is demonstrated, with their long half-lives and good expected tolerability, we anticipate they will offer tremendous value for clinicians aiming to relieve the burden of individuals with episodic or CM.

STATEMENT OF AUTHORSHIP

Category 1

(a) Conception and Design
Marcelo E. Bigal; Sarah Walter; Alan M. Rapoport

(b) Acquisition of Data
n/a review paper

(c) Analysis and Interpretation of Data
n/a review paper

Category 2

(a) Drafting the Manuscript
Marcelo E. Bigal

(b) Revising It for Intellectual Content
Marcelo E. Bigal; Sarah Walter; Alan M. Rapoport

Category 3

(a) Final Approval of the Completed Manuscript
Marcelo E. Bigal; Sarah Walter; Alan M. Rapoport

REFERENCES

1. Terenghi G, Polak JM, Ghatei MA, et al. Distribution and origin of calcitonin gene-related peptide (CGRP) immunoreactivity in the sensory innervation of the mammalian eye. *J Comp Neurol*. 1985;233:506-516.
2. Alevizaki M, Shiraishi A, Rassool FV, Ferrier GJ, MacIntyre I, Legon S. The calcitonin-like sequence of the beta CGRP gene. *FEBS Lett*. 1986;206:47-52.
3. Raddant AC, Russo AF. Calcitonin gene-related peptide in migraine: Intersection of peripheral inflammation and central modulation. *Expert Rev Mol Med*. 2011;13:e36.
4. Noguchi K, Senba E, Morita Y, Sato M, Tohyama M. Alpha-CGRP and beta-CGRP mRNAs are differentially regulated in the rat spinal cord and dorsal root ganglion. *Brain Res Mol Brain Res*. 1990;7:299-304.
5. Tippins JR, Di Marzo V, Panico M, Morris HR, MacIntyre I. Investigation of the structure/activity relationship of human calcitonin gene-related peptide (CGRP). *Biochem Biophys Res Commun*. 1986;134:1306-1311.
6. Juaneda C, Dumont Y, Quirion R. The molecular pharmacology of CGRP and related peptide receptor subtypes. *Trends Pharmacol Sci*. 2000;21:432-438.
7. Recober A, Russo AF. Calcitonin gene-related peptide: An update on the biology. *Curr Opin Neurol*. 2009;22:241-246.
8. Emeson RB, Hedjran F, Yeakley JM, Guise JW, Rosenfeld MG. Alternative production of calcitonin and CGRP mRNA is regulated at the calcitonin-specific splice acceptor. *Nature*. 1989; 341:76-80.
9. van Rossum D, Hanisch UK, Quirion R. Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors. *Neurosci Biobehav Rev*. 1997;21: 649-678.
10. Edvinsson L, Goadsby PJ. Extracerebral manifestations in migraine. A peptidergic involvement? *J Intern Med*. 1990;228:299-304.
11. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol*. 1990;28:183-187.
12. Peroutka SJ. Clinical trials update – 2012: Year in review. *Headache*. 2013;53:177-180.
13. Silberstein SD. Emerging target-based paradigms to prevent and treat migraine. *Clin Pharmacol Ther*. 2013;93:78-85.
14. Lundberg JM, Franco-Cereceda A, Hua X, Hokfelt T, Fischer JA. Co-existence of substance P and calcitonin gene-related peptide-like immunoreactivities in sensory nerves in relation to cardiovascular and bronchoconstrictor effects of capsaicin. *Eur J Pharmacol*. 1985;108:315-319.

15. Lundberg JM, Franco-Cereceda A, Alving K, Delay-Goyet P, Lou YP. Release of calcitonin gene-related peptide from sensory neurons. *Ann N Y Acad Sci*. 1992;657:187-193.
16. Mulderry PK, Ghatei MA, Spokes RA, et al. Differential expression of alpha-CGRP and beta-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. *Neuroscience*. 1988; 25:195-205.
17. Feuerstein G, Willette R, Aiyar N. Clinical perspectives of calcitonin gene related peptide pharmacology. *Can J Physiol Pharmacol*. 1995;73:1070-1074.
18. Poyner D. Pharmacology of receptors for calcitonin gene-related peptide and amylin. *Trends Pharmacol Sci*. 1995;16:424-428.
19. Toda M, Suzuki T, Hosono K, et al. Neuronal system-dependent facilitation of tumor angiogenesis and tumor growth by calcitonin gene-related peptide. *Proc Natl Acad Sci U S A*. 2008;105:13550-13555.
20. Allen DM, Chen LE, Seaber AV, Urbaniak JR. Calcitonin gene-related peptide and reperfusion injury. *J Orthop Res*. 1997;15:243-248.
21. Kallner G, Gonon A, Franco-Cereceda A. Calcitonin gene-related peptide in myocardial ischaemia and reperfusion in the pig. *Cardiovasc Res*. 1998; 38:493-499.
22. Lynch JJ Jr, Detwiler TJ, Kane SA, Regan CP. Effect of calcitonin gene-related peptide receptor antagonism on the systemic blood pressure responses to mechanistically diverse vasomodulators in conscious rats. *J Cardiovasc Pharmacol*. 2010;56:518-525.
23. Supowit SC, Ethridge RT, Zhao H, Katki KA, Dipette DJ. Calcitonin gene-related peptide and substance P contribute to reduced blood pressure in sympathectomized rats. *Am J Physiol Heart Circ Physiol*. 2005;289:H1169-H1175.
24. Brain SD, Grant AD. Vascular actions of calcitonin gene-related peptide and adrenomedullin. *Physiol Rev*. 2004;84:903-934.
25. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I. Calcitonin gene-related peptide is a potent vasodilator. *Nature*. 1985;313:54-56.
26. Sinclair SR, Kane SA, Van der Schueren BJ, et al. Inhibition of capsaicin-induced increase in dermal blood flow by the oral CGRP receptor antagonist, telcagepant (MK-0974). *Br J Clin Pharmacol*. 2010;69:15-22.
27. Goadsby PJ, Edvinsson L, Ekman R. Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigeminovascular system. *Ann Neurol*. 1988;23: 193-196.
28. Denekas T, Troltzsch M, Vater A, Klussmann S, Messlinger K. Inhibition of stimulated meningeal blood flow by a calcitonin gene-related peptide binding mirror-image RNA oligonucleotide. *Br J Pharmacol*. 2006;148:536-543.
29. Juhasz G, Zsombok T, Jakab B, Nemeth J, Szolcsanyi J, Bagdy G. Sumatriptan causes parallel decrease in plasma calcitonin gene-related peptide (CGRP) concentration and migraine headache during nitroglycerin induced migraine attack. *Cephalalgia*. 2005;25:179-183.
30. Juhasz G, Zsombok T, Modos EA, et al. NO-induced migraine attack: Strong increase in plasma calcitonin gene-related peptide (CGRP) concentration and negative correlation with platelet serotonin release. *Pain*. 2003;106:461-470.
31. Hansen JM, Hauge AW, Olesen J, Ashina M. Calcitonin gene-related peptide triggers migraine-like attacks in patients with migraine with aura. *Cephalalgia*. 2010;30:1179-1186.
32. Ashina M, Bendtsen L, Jensen R, Schifter S, Jansen-Olesen I, Olesen J. Plasma levels of calcitonin gene-related peptide in chronic tension-type headache. *Neurology*. 2000;55:1335-1340.
33. Lassen LH, Haderslev PA, Jacobsen VB, Iversen HK, Sperling B, Olesen J. CGRP may play a causative role in migraine. *Cephalalgia*. 2002;22:54-61.
34. Zhang JF, Liu J, Liu XZ, Sheng SL, Zhang WJ, Chen YJ. The effect of calcitonin gene-related peptide on arrhythmia caused by adenosine diphosphate and desacetyldigilanide-C in rats. *Int J Cardiol*. 1991;33:43-46.
35. Atkins BZ, Silvestry SC, Samy RN, Shah AS, Sabiston DC Jr, Glower DD. Calcitonin gene-related peptide enhances the recovery of contractile function in stunned myocardium. *J Thorac Cardiovasc Surg*. 2000;119:1246-1254.
36. Zhou ZH, Peng J, Ye F, Li NS, Deng HW, Li YJ. Delayed cardioprotection induced by nitroglycerin is mediated by alpha-calcitonin gene-related peptide. *Naunyn Schmiedebergs Arch Pharmacol*. 2002;365:253-259.
37. Chan KY, Edvinsson L, Eftekhari S, et al. Characterization of the calcitonin gene-related peptide

receptor antagonist telcagepant (MK-0974) in human isolated coronary arteries. *J Pharmacol Exp Ther*. 2010;334:746-752.
38. Lynch JJ, Shen YT, Pittman TJ, et al. Effects of the prototype serotonin 5-HT(1B/1D) receptor agonist sumatriptan and the calcitonin gene-related peptide (CGRP) receptor antagonist CGRP(8-37) on myocardial reactive hyperemic response in conscious dogs. *Eur J Pharmacol*. 2009;623:96-102.
39. Petersen KA, Birk S, Lassen LH, et al. The CGRP-antagonist, BIBN4096BS does not affect cerebral or systemic haemodynamics in healthy volunteers. *Cephalalgia*. 2005;25:139-147.
40. Verheggen R, Bumann K, Kaumann AJ. BIBN4096BS is a potent competitive antagonist of the relaxant effects of alpha-CGRP on human temporal artery: Comparison with CGRP(8-37). *Br J Pharmacol*. 2002;136:120-126.
41. Van der Schueren BJ, Blanchard R, Murphy MG, et al. The potent calcitonin gene-related peptide receptor antagonist, telcagepant, does not affect nitroglycerin-induced vasodilation in healthy men. *Br J Clin Pharmacol*. 2011;71:708-717.
42. Chaitman BR, Ho AP, Behm MO, et al. A randomized, placebo-controlled study of the effects of telcagepant on exercise time in patients with stable angina. *Clin Pharmacol Ther*. 2012;91:459-466.
43. Conolan S, Taylor DA. Antagonism by some ergot derivatives of 5-HT-induced vasoconstriction. *Eur J Pharmacol*. 1986;123:299-302.
44. Maassen VanDenBrink A, Bax WA, Ferrari MD, Zijlstra FJ, Bos E, Saxena PR. Augmented contraction of the human isolated coronary artery by sumatriptan: A possible role for endogenous thromboxane. *Br J Pharmacol*. 1996;119:855-862.
45. Longmore J, Hargreaves RJ, Boulanger CM, et al. Comparison of the vasoconstrictor properties of the 5-HT1D-receptor agonists rizatriptan (MK-462) and sumatriptan in human isolated coronary artery: Outcome of two independent studies using different experimental protocols. *Funct Neurol*. 1997;12:3-9.
46. Li L, Wang X, Yu LC. Involvement of opioid receptors in the CGRP-induced antinociception in the nucleus accumbens of rats. *Brain Res*. 2010;1353:53-59.
47. Yu LC, Weng XH, Wang JW, Lundeberg T. Involvement of calcitonin gene-related peptide and its receptor in anti-nociception in the periaqueductal grey of rats. *Neurosci Lett*. 2003;349:1-4.
48. Benarroch EE. CGRP: Sensory neuropeptide with multiple neurologic implications. *Neurology*. 2011;77:281-287.
49. Messlinger K, Hanesch U, Kurosawa M, Pawlak M, Schmidt RF. Calcitonin gene related peptide released from dural nerve fibers mediates increase of meningeal blood flow in the rat. *Can J Physiol Pharmacol*. 1995;73:1020-1024.
50. Unger JW, Lange W. Immunohistochemical mapping of neurophysins and calcitonin gene-related peptide in the human brainstem and cervical spinal cord. *J Chem Neuroanat*. 1991;4:299-309.
51. Lennerz JK, Ruhle V, Ceppa EP, et al. Calcitonin receptor-like receptor (CLR), receptor activity-modifying protein 1 (RAMP1), and calcitonin gene-related peptide (CGRP) immunoreactivity in the rat trigeminovascular system: Differences between peripheral and central CGRP receptor distribution. *J Comp Neurol*. 2008;507:1277-1299.
52. Messlinger K, Lennerz JK, Eberhardt M, Fischer MJ. CGRP and NO in the trigeminal system: Mechanisms and role in headache generation. *Headache*. 2012;52:1411-1427.
53. Eftekhari S, Edvinsson L. Possible sites of action of the new calcitonin gene-related peptide receptor antagonists. *Ther Adv Neurol Disord*. 2010;3:369-378.
54. Vause CV, Durham PL. Calcitonin gene-related peptide differentially regulates gene and protein expression in trigeminal glia cells: Findings from array analysis. *Neurosci Lett*. 2010;473:163-167.
55. Ceruti S, Villa G, Fumagalli M, et al. Calcitonin gene-related peptide-mediated enhancement of purinergic neuron/glia communication by the algogenic factor bradykinin in mouse trigeminal ganglia from wild-type and R192Q Cav2.1 Knock-in mice: Implications for basic mechanisms of migraine pain. *J Neurosci*. 2011;31:3638-3649.
56. Russo AF, Kuburas A, Kaiser EA, Raddant AC, Recober A. A potential preclinical migraine model: CGRP-sensitized mice. *Mol Cell Pharmacol*. 2009;1:264-270.
57. Markowitz S, Saito K, Buzzi MG, Moskowitz MA. The development of neurogenic plasma extravasation in the rat dura mater does not depend upon the degranulation of mast cells. *Brain Res*. 1989;477:157-165.

58. Eftekhari S, Edvinsson L. Calcitonin gene-related peptide (CGRP) and its receptor components in human and rat spinal trigeminal nucleus and spinal cord at C1-level. *BMC Neurosci*. 2011;12:112.
59. Mathew R, Andreou AP, Chami L, et al. Immunohistochemical characterization of calcitonin gene-related peptide in the trigeminal system of the familial hemiplegic migraine 1 knock-in mouse. *Cephalalgia*. 2011;31:1368-1380.
60. Goadsby PJ. Neurovascular headache and a midbrain vascular malformation: Evidence for a role of the brainstem in chronic migraine. *Cephalalgia*. 2002;22:107-111.
61. Bahra A, Matharu MS, Buchel C, Frackowiak RS, Goadsby PJ. Brainstem activation specific to migraine headache. *Lancet*. 2001;357:1016-1017.
62. Moskowitz MA. Neurogenic inflammation in the pathophysiology and treatment of migraine. *Neurology*. 1993;43(6 Suppl. 3):S16-S20.
63. Williamson DJ, Hargreaves RJ. Neurogenic inflammation in the context of migraine. *Microsc Res Tech*. 2001;53:167-178.
64. Sun RQ, Lawand NB, Willis WD. The role of calcitonin gene-related peptide (CGRP) in the generation and maintenance of mechanical allodynia and hyperalgesia in rats after intradermal injection of capsaicin. *Pain*. 2003;104:201-208.
65. Bigal ME, Ferrari M, Silberstein SD, Lipton RB, Goadsby PJ. Migraine in the triptan era: Lessons from epidemiology, pathophysiology, and clinical science. *Headache*. 2009;49(Suppl. 1):S21-S33.
66. Ho TW, Edvinsson L, Goadsby PJ. CGRP and its receptors provide new insights into migraine pathophysiology. *Nat Rev Neurol*. 2010;6:573-582.
67. Morara S, Rosina A, Provini L, Forloni G, Caretti A, Wimalawansa SJ. Calcitonin gene-related peptide receptor expression in the neurons and glia of developing rat cerebellum: An autoradiographic and immunohistochemical analysis. *Neuroscience*. 2000;100:381-391.
68. Pagani F, Guidobono F, Netti C, Re F, Pecile A. Age-related increase in CGRP binding site densities in rat cerebellum. *Pharmacol Res*. 1989; 21(Suppl. 1):105-106.
69. Noseda R, Burstein R. Advances in understanding the mechanisms of migraine-type photophobia. *Curr Opin Neurol*. 2011;24:197-202.
70. Recober A, Kuburas A, Zhang Z, Wemmie JA, Anderson MG, Russo AF. Role of calcitonin gene-related peptide in light-aversive behavior: Implications for migraine. *J Neurosci*. 2009;29:8798-8804.
71. Seybold VS, Galeazza MT, Garry MG, Hargreaves KM. Plasticity of calcitonin gene related peptide neurotransmission in the spinal cord during peripheral inflammation. *Can J Physiol Pharmacol*. 1995;73:1007-1014.
72. Bird GC, Han JS, Fu Y, Adwanikar H, Willis WD, Neugebauer V. Pain-related synaptic plasticity in spinal dorsal horn neurons: Role of CGRP. *Mol Pain*. 2006;2:31.
73. Galeazza MT, Garry MG, Yost HJ, Strait KA, Hargreaves KM, Seybold VS. Plasticity in the synthesis and storage of substance P and calcitonin gene-related peptide in primary afferent neurons during peripheral inflammation. *Neuroscience*. 1995;66: 443-458.
74. Moulton EA, Schmahmann JD, Becerra L, Borsook D. The cerebellum and pain: Passive integrator or active participator? *Brain Res Rev*. 2010;65:14-27.
75. Kruit MC, Launer LJ, Ferrari MD, van Buchem MA. Infarcts in the posterior circulation territory in migraine. The population-based MRI CAMERA study. *Brain*. 2005;128(Pt 9):2068-2077.
76. Kruit MC, van Buchem MA, Hofman PA, et al. Migraine as a risk factor for subclinical brain lesions. *JAMA*. 2004;291:427-434.
77. Olesen J, Diener HC, Husstedt IW, et al. Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. *N Engl J Med*. 2004;350:1104-1110.
78. Ho TW, Mannix LK, Fan X, et al. Randomized controlled trial of an oral CGRP receptor antagonist, MK-0974, in acute treatment of migraine. *Neurology*. 2008;70:1304-1312.
79. Ho TW, Ferrari MD, Dodick DW, et al. Efficacy and tolerability of MK-0974 (telcagepant), a new oral antagonist of calcitonin gene-related peptide receptor, compared with zolmitriptan for acute migraine: A randomised, placebo-controlled, parallel-treatment trial. *Lancet*. 2008;372:2115-2123.
80. Hoffmann J, Goadsby PJ. New agents for acute treatment of migraine: CGRP receptor antagonists, iNOS inhibitors. *Curr Treat Options Neurol*. 2012; 14:50-59.
81. Salvatore CA, Moore EL, Calamari A, et al. Pharmacological properties of MK-3207, a potent and orally active calcitonin gene-related peptide

receptor antagonist. *J Pharmacol Exp Ther*. 2010; 333:152-160.
82. Hewitt DJ, Aurora SK, Dodick DW, et al. Randomized controlled trial of the CGRP receptor antagonist MK-3207 in the acute treatment of migraine. *Cephalalgia*. 2011;31:712-722.
83. Pettypiece S. Merck halts testing of migraine drug on liver safety (Update2). 2009. Available at: http://www.bloomberg.com/apps/news?pid=newsarchive&sid=aKxzpNBZ2cbA (accessed April 4, 2013).
84. Diener HC, Barbanti P, Dahlof C, Reuter U, Habeck J, Podhorna J. BI 44370 TA, an oral CGRP antagonist for the treatment of acute migraine attacks: Results from a phase II study. *Cephalalgia*. 2011;31:573-584.
85. Connor KM, Aurora SK, Loeys T, et al. Long-term tolerability of telcagepant for acute treatment of migraine in a randomized trial. *Headache*. 2011;51: 73-84.
86. Health NIo. Study to Evaluate the PK of BMS-927711 in Patient With Migraine During Acute Migraine and Non-migraine Condition. 2013. Available at: http://clinicaltrial.gov/ct2/show/NCT01445067?term=CGRP&recr=Open&rank=4 (accessed April 5, 2013).
87. A dose-finding study of MK-1602 in the treatment of acute migraine (MK-1602-006 AM1). 2013. Available at: http://clinicaltrial.gov/ct2/show/NCT01613248?term=MK1602&rank=2 (accessed April 10, 2013).
88. Miller RA, Maloney DG, Warnke R, Levy R. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. *N Engl J Med*. 1982;306: 517-522.
89. Mould DR, Sweeney KR. The pharmacokinetics and pharmacodynamics of monoclonal antibodies–mechanistic modeling applied to drug development. *Curr Opin Drug Discov Devel*. 2007;10:84-96.
90. Harding FA, Stickler MM, Razo J, DuBridge RB. The immunogenicity of humanized and fully human antibodies: Residual immunogenicity resides in the CDR regions. *Mabs*. 2010;2:256-265.
91. Tsurushita N, Hinton PR, Kumar S. Design of humanized antibodies: From anti-Tac to Zenapax. *Methods*. 2005;36:69-83.
92. Hwang WY, Foote J. Immunogenicity of engineered antibodies. *Methods*. 2005;36:3-10.
93. Wu H, Dall'Acqua WF. Humanized antibodies and their applications. *Methods*. 2005;36:1-2.
94. Services USDoHaH, Administration FaD, (CDER) CfDEaR, (CBER) CfBEaR. Guidance for industry. M3(R2) nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. 1994. Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073246.pdf (accessed April 24, 2013).
95. Baumann A. Early development of therapeutic biologics–pharmacokinetics. *Curr Drug Metab*. 2006;7:15-21.
96. Bussiere JL. General toxicity testing and immunotoxicity testing for biopharmaceuticals. In: Cavagnaro JA, ed. *Preclinical Safety Evaluation of Biopharmaceuticals*. New York: Wiley; 2008:125-155.
97. Services UDoHaH, Administration FaD, (CDER) CfDEaR, (CBER) CfBEaR. Guidance for industry, S6 Preclinical safety evaluation of biotechnology-derived pharmaceuticals, 1997. Available at: http://www.fda.gov/downloads/regulatoryinformation/guidances/ucm129171.pdf (accessed April 20, 2013).
98. Vargas HM, Bass AS, Breidenbach A, et al. Scientific review and recommendations on preclinical cardiovascular safety evaluation of biologics. *J Pharmacol Toxicol Methods*. 2008;58:72-76.
99. clinicaltrial.gov. A study of LY2951742 in healthy volunteers. 2013. Available at: http://www.clinicaltrials.gov/ct2/show/NCT01337596?term=LY2951742&rank=1 (accessed April 10, 2013).
100. A study of LY2951742 in patients with migraine. 2013. Available at: http://www.clinicaltrials.gov/ct2/show/NCT01625988?term=LY2951742&rank=2 (accessed April 10, 2013).
101. Safety, efficacy and pharmacokinetics of ALD403. 2013. Available at: http://clinicaltrials.gov/ct2/show/NCT01772524?term=ald403&rank=2 (accessed April 10, 2013).
102. Amgen – science – pipeline. 2013. Available at: http://www.amgen.com/science/pipe.html (accessed April 10, 2013).
103. Ascending single doses of AMG 334 in healthy subjects and migraine patients. 2013. Available at: http://clinicaltrial.gov/ct2/show/NCT01688739?term=AMG334&rank=1 (accessed April 10, 2013).
104. Ascending multiple-doses of AMG 334 in healthy subjects and in migraine patients. 2013. Available at: http://clinicaltrial.gov/ct2/show/NCT01723514?term=AMG334&rank=2 (accessed April 10, 2013).

105. Phase 1 studies of LBR-101 (PF-04427429) listed at clinicaltrials.gov. 2013. Available at: http://clinicaltrials.gov/ct2/results?term=PF-04427429&Search=Search (accessed April 10, 2013).
106. Bigal ME, Buse DC, Chen YT, et al. Rates and predictors of starting a triptan: Results from the american migraine prevalence and prevention study. *Headache*. 2010;50:1440-1448.
107. Bigal ME, Borucho S, Serrano D, Lipton RB. The acute treatment of episodic and chronic migraine in the USA. *Cephalalgia*. 2009;29:891-897.
108. Ferrari MD, Goadsby PJ, Roon KI, Lipton RB. Triptans (serotonin, 5-HT1B/1D agonists) in migraine: Detailed results and methods of a meta-analysis of 53 trials. *Cephalalgia*. 2002;22:633-658.
109. Bigal ME, Serrano D, Buse D, Scher A, Stewart WF, Lipton RB. Acute migraine medications and evolution from episodic to chronic migraine: A longitudinal population-based study. *Headache*. 2008;48:1157-1168.
110. Bigal ME, Kurth T, Santanello N, et al. Migraine and cardiovascular disease: A population-based study. *Neurology*. 2010;74:628-635.
111. Lipton RB, Bigal ME, Diamond M, et al. Migraine prevalence, disease burden, and the need for preventive therapy. *Neurology*. 2007;68:343-349.
112. Silberstein SD, Dodick D, Kesslick J. Removing barriers to appropriate migraine treatment: Formulary limitations and triptan package size. *Headache*. 2005;45:1250-1254.
113. Lipton RB, Varon SF, Grosberg B, et al. OnabotulinumtoxinA improves quality of life and reduces impact of chronic migraine. *Neurology*. 2011;77:1465-1472.
114. Evans RW. A rational approach to the management of chronic migraine. *Headache*. 2013;53:168-176.
115. Connor KM, Shapiro RE, Diener HC, et al. Randomized, controlled trial of telcagepant for the acute treatment of migraine. *Neurology*. 2009;73:970-977.