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CGRP as the target of new migraine therapies: A successful translation from bench to clinic

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Abstract

The treatment of migraine, a disabling chronic neurological pain disorder, is on the cusp of a new era with the development of novel drugs that target the trigeminal sensory neuropeptide, calcitonin gene-related peptide (CGRP) or its receptor. Based on successful clinical trials, it is anticipated that a number of these drugs will be approved in 2018 and 2019 for use in migraine headache. CGRP-related therapies represent a significant improvement over existing drugs in that they are designed specifically to act on the trigeminal pain system, are more specific and show little-to-no side-effects. CGRP receptor antagonists such as ubrogepant are effective in acute relief of migraine headache, whereas monoclonal antibodies against CGRP (eptinezumab, fremanezumab and galcanezumab) or the CGRP receptor (erenumab) are efficacious in prevention of migraine attacks. As these drugs come into clinical use, there will be increased interest in CGRP and its involvement in headache pain. Thus it is timely to provide an overview of where and how these drugs work based on the knowledge gained over the past 30 years of CGRP research. This review describes CGRP signaling components targeted by the new drugs, summarizes key clinical evidence pointing to the important role of CGRP in migraine headache and synthesizes what is known about the role of CGRP in the trigeminal system. The recent findings regarding CGRP and the effectiveness of CGRP-targeted therapies in migraine offer new insight into the central role of the trigeminal ganglion in the pathophysiology of migraine.

Introduction

Long-suffering migraine patients will soon have access to a novel group of medications that are effective in treating or preventing the debilitating, painful headaches associated with this disorder. The new therapies all work by interfering with a potent neuropeptide in sensory nerves, calcitonin gene-related peptide (CGRP). This is a unique strategy for treating migraine, a chronic primary headache disorder that afflicts almost 15% of the population worldwide[1]. Previous treatment options have not met the challenge of alleviating the personal and economic burden of migraine, which is the leading cause of neurological disability and one of the five main causes of long-term disability overall [1,2]. Inadequate efficacy, tolerability and patient adherence have limited the effectiveness of the older and current migraine prevention [3-5]. Drugs that are currently taken for prophylaxis are not specific for migraine but include anti-epileptic drugs, β-blockers and anti-depressants [6,7].

Migraine is a chronic, complex neurological disorder that manifests as recurrent attacks of moderate to severe headache pain lasting 4-72 hours. Typically, the headache is unilateral, with a pulsating quality, and it is aggravated by routine physical activity and associated with nausea and/or sensitivity to light and sound [8]. In migraine with aura, the headache phase is preceded by reversible focal neurological symptoms, often visual or sensory, that usually develop gradually over 5–20 minutes and last for less than 60 minutes. Migrainers who experience frequent attacks are considered to have either episodic (1-14 migraine days/month or fewer) or chronic ($\geq$ 15 migraine days/month) migraine.

All CGRP-targeted therapies tested to date have consistently shown positive results in treating migraine (Table 1) [9,10]. CGRP receptor antagonists provide relief from an acute headache attack, either with or without aura. Monoclonal antibodies against either CGRP or its

receptor are particularly effective for prevention of episodic or chronic migraine. The drugs currently being developed are well-tolerated with little adverse effects. Thus, identification of CGRP as a specific therapeutic target for migraine has resulted in a major breakthrough in providing relief for this common disorder. The translation of CGRP therapies to the clinic has been built on a long journey of discovery that is outlined in the timeline shown in Box 1. The purpose of this review is to summarize what has been learned regarding the role of CGRP in migraine and the trigeminovascular pain pathway, and discuss the sites and mechanisms of action of the new CGRP-based migraine therapies.

Components of CGRP transmission relevant to migraine therapies

CGRP

CGRP is a peptide neurotransmitter that is located in peripheral sensory neurons and numerous sites throughout the central nervous system. Studies of the trigeminovascular system have provided much of what is known about the role of CGRP in the cranial sensory nerves involved in migraine [9,11]. The components of CGRP transmission that are targeted by migraine therapies are illustrated using a schematic of the trigeminal innervation of a cerebrovascular smooth muscle cell (Fig. 1).

The α form of CGRP is a 37-amino acid peptide (Fig. 1A) that is synthesized in neurons by tissue-specific splicing of mRNA transcribed from the calcitonin/CGRP gene located on chromosome 11 [12,13]. CGRP is generated by cleavage of a pro-peptide precursor and packaged into dense core vesicles for transport to axon terminals and other release sites within the neuron.

The first antibodies against CGRP were made shortly after discovery of the peptide in 1982 [13]. They were used initially for radioimmunoassays, in combination with HPLC (high-performance liquid chromatography), to measure CGRP levels and for tissue localization of the

peptide using immunohistochemistry [14,15]. More recently, specific antibodies are being used to bind CGRP and inhibit its action (Fig. 1C). Several humanized monoclonal CGRP antibodies (galcanezumab, eptinezumab, fremanezumab) are currently undergoing clinical trials and have been shown to be effective for prevention in episodic and chronic migraine (Table 1) [4,9,10,16,17].

CGRP Release

In CGRP nerves, the presynaptic terminals take the form of focal swellings, called axonal varicosities, which occur at regular intervals along the axon like a strand of pearls. Upon nerve stimulation, CGRP is released from its storage vesicles via calcium-dependent exocytosis. Capsaicin, a component of chili peppers, also causes release of αCGRP; this compound has been useful as an experimental tool to release and ultimately deplete the peptide from CGRP nerves [18,19]. Presynaptic receptors located on trigeminal neurons regulate CGRP release. Presynaptic serotonin 5-HT_{1B} and 5-HT_{1D} receptors inhibit CGRP release (Fig. 1C) [20]; they are the target for therapeutic effects of the triptans, e.g. sumatriptan, in relief of migraine [3,20-23]. Recently, a third subtype of presynaptic serotonin receptor, 5-HT_{1F}, has become a target of interest in migraine [3]. Activation of this receptor also suppresses trigeminal release of CGRP [24], and recent clinical studies show that a 5-HT_{1F} agonist, lasmiditan, has acute anti-migraine efficacy [25].

Following release, CGRP is broken down by metalloproteases [26]. Amidation at the C-terminus helps protect the peptide and increase its half-life. This property allows CGRP to spread to receptor targets beyond the release site, in what is known as volume transmission [27]. CGRP in blood is generally attributed to spillover from sites of neuronal release, as was demonstrated for release of trigeminal CGRP into the rat jugular vein [28]. Release of CGRP after stimulation of the trigeminal ganglion in humans also has been measured in blood collected from

the external jugular vein draining the extracerebral tissues [29] (Fig. 2A). Human plasma levels of CGRP are usually within the low picomolar range. The plasma half-life of CGRP in humans was estimated following CGRP infusion to be 7 min for a fast decay phase and 26 min for a slower phase of decay [30].

CGRP Receptor

The CGRP receptor is a complex of several proteins that are required for the ligand specificity and function of this receptor (Fig. 1B). Central to the complex is a G protein-coupled receptor, calcitonin receptor-like receptor (CLR). CLR is a member of the secretin receptor family (Class B GPCR), and it is a required element in receptors for CGRP, amylin and adrenomedullin (AM1 and AM2). In order to create a functional membrane receptor with specific affinity for CGRP, CLR must form a heterodimer with a specific receptor activity modifying protein (RAMP), RAMP1 [31]. RAMPs are single transmembrane-spanning proteins that alter the pharmacology, functionality and cell trafficking of specific GPCRs. The ligand-binding domain of the CGRP receptor is located at the interface between the RAMP1 and CLR proteins [32,33]. Thus, co-expression of both CLR and RAMP1 is necessary for a cell to respond to CGRP.

The CGRP receptor complex also includes two cytoplasmic proteins that associate with the CLR-RAMP1 heterodimer to mediate signal transduction (Fig. 1B). CLR is coupled to a G-protein containing the Gs alpha subunit (Gαs) that activates adenylyl cyclase (AC) and cAMP-dependent signaling pathways [12,34]. In addition, the CGRP receptor associates with a receptor coupling protein (RCP) that amplifies G-protein activation and is important for optimal signal transduction [34]. Receptor-mediated increases in intracellular cAMP activate protein kinase A

(PKA), resulting in the phosphorylation of multiple downstream targets, including potassium-sensitive ATP channels (K_{ATP} channels), extracellular signal-related kinases (ERKs), or transcription factors such as cAMP response element-binding protein (CREB). In cerebrovascular smooth muscle (Fig. 1C), elevation of cAMP by CGRP results in vasorelaxation and dilation of the blood vessel [35].

An important feature of CGRP signaling is the regulation and desensitization of the receptor following agonist activation [36]. After CGRP binds its receptor, the CLR component is rapidly phosphorylated and the receptor is subsequently internalized via recruitment of β-arrestins. Transient stimulation by CGRP induces internalization of the receptor to endosomes that allows rapid recycling back to the plasma membrane. Chronic exposure to CGRP, however, initiates an internalization process that trafficks the receptor to lysosomes for degradation.

CGRP Receptor Antagonists

A number of small molecule antagonists have been identified that potently and selectively block CGRP responses in both experimental and clinical studies [37,38]. Although chemically unrelated, this class of antagonists is collectively called "gepants" due to their common mode of action (Fig. 1C). All gepants tested to date are efficacious in migraine patients [39-41]. Interestingly, a common feature of the clinically-active gepants is their high affinity (picomolar) for CGRP receptors of humans and non-human primates relative to other species. This is due to a species-specific residue located at the interface between the RAMP1 and CLR proteins, indicating this region as the site of antagonist binding [32].

Olcegepant was the first non-peptide CGRP receptor antagonist to be discovered [42]. It potently blocked the binding of CGRP to its receptor in both cells and tissue preparations and

produced a rightward shift of CGRP concentration-response curves in signaling (cAMP production) and functional (vasodilation) assays [42,43]. Olcegepant also blocked CGRP responses *in vivo* [44] and was formulated for intravenous testing in humans [45]. Subsequently, other gepants have been developed that exhibit increased potency and good oral bioavailabilty, e.g., telcagepant [46,47], ubrogepant [48], MK-3207, BI 44370 TA, and MK-8031 [4] . However the clinical development of a number of gepants has been terminated, in part, due to potential for liver toxicity [5,49] (Timeline Box1).

CGRP Receptor Antibody

A novel approach to blocking CGRP receptors has been the development of a monoclonal antibody targeted to the receptor (Fig. 1C). Erenumab (AMG 334) is a human monoclonal antibody that was raised against a fusion protein of the extracellular domains of human CLR and RAMP1 that comprise the CGRP binding pocket [50]. This antibody was shown to be 5000-fold more selective for the human CGRP receptor than other related receptors in the calcitonin receptor family. Erenumab binds human CGRP receptors with high affinity, and it fully antagonizes CGRP responses *in vitro* and *in vivo* [50]. Clinical trials show erenumab to be effective prophylactic therapy in episodic and chronic migraine patients [4,38].

Clinical Evidence for a Role of CGRP in Migraine

Because trigeminal sensory nerves play a key role in primary headache disorders, the discovery of CGRP in the trigeminovascular system immediately suggested that this peptide could be of considerable importance in migraine pathology [51]. Moreover, the potent vasodilatory effects of CGRP in cerebral arteries [35] fit the prevailing view that cerebral vasodilation was an

important component of headache. The presence of CGRP in human trigeminal ganglia and cerebral arteries was soon confirmed [15,52]. Interestingly, peptide levels were highest in younger subjects (20-40 yrs old) and declined with age , consistent with the age-related pattern of headache attacks observed in migraine patients. Important milestones in the story of CGRP and migraine are indicated in the Timeline (Box1) and discussed below. Together these findings unequivocally support a major role for CGRP in the symptoms of migraine and underscore the effectiveness of blocking CGRP signaling to abort and prevent painful migraine attacks.

Elevated CGRP Release in Migraine and Primary Headache Disorders

In 1990, Goadsby *et al.* made the seminal observation that CGRP is uniquely released during an acute migraine attack [53]. Blood samples taken from the external jugular vein of patients showed elevated CGRP levels during the headache (Fig. 2A), but no change in the levels of other neuropeptides associated with trigeminal and autonomic nerves. CGRP was released during attacks of migraine that occurred either with or without aura. Elevated levels of CGRP also were found during attacks in other primary headache disorders such as cluster headache and chronic paroxysmal headache [54,55]. In the latter two conditions, in contrast to migraine, vasoactive intestinal peptide (VIP) was released in addition to CGRP (Fig. 2A). Further studies of migraine patients have confirmed that CGRP levels are elevated in plasma as well as saliva samples [55-57]. There is interest in using these values for diagnostic purposes in migraine; however, the instability and short half-life of the peptide is a complicating factor in obtaining reliable measurements. For example, in contrast to the clear results from blood sampling in the external jugular vein during migraine (Fig. 2A), no change in CGRP was detected in samples taken at the same time from the peripheral circulation [53].

Additional support for the role of CGRP in headache comes from several clinical studies in which exogenous CGRP was infused i.v. into patients prone to migraine attacks [58,59]. This stimulus induced a lasting, migraine-like headache, suggesting that CGRP may play a causal role in migraine symptoms.

Triptan Inhibition of CGRP Release during Migraine Attacks

Triptans have been the mainstay of acute migraine therapy since sumatriptan was first shown to relieve migraine symptoms in the early 1990's [3]. These drugs are partial agonists at serotonin 5-HT_{1B} and 5-HT_{1D} receptors, and they were originally thought to relieve migraine through their vasoconstrictor effects and/or regulation of trigeminal transmitter release. In 1993-1994, Goadsby and Edvinsson tested whether sumatriptan would affect extracranial plasma levels of CGRP in patients during the headache phase of migraine or cluster headache [20,21,23,54]. They found sumatriptan prevented the increase in plasma CGRP at the same time it aborted the headache attack (Fig. 2A). This striking finding validated the key therapeutic mechanism for triptan drugs and underscored the role of CGRP in migraine.

Efficacy of Gepants in Migraine

All gepants that have been tested in clinical trials significantly reduce headache pain and other symptoms associated with an acute migraine attack (Fig. 2B) [38]. This consistent clinical outcome validates the therapeutic strategy of blocking CGRP receptors for relief from migraine. The first proof-of-concept study for this approach was a small, double-blind, randomized trial in 2004 that showed olcegepant, i.v., significantly alleviated symptoms during a migraine attack [45]. 66% of the patients with moderate-to-severe headache achieved the primary efficacy end point of pain relief, defined as having no or mild headache, at two hours after dosing (Fig. 2B). Nausea,

photophobia, phonophobia, and functional capacity also improved in parallel with the extent of response to treatment. The efficacy of olcegepant was comparable to that of triptans, but no cardiovascular or hemodynamic symptoms were observed. The adverse effects limit the use of triptans in patients with cardiovascular pathologies [3]; but CGRP antagonists do not cause vasoconstriction [38], making them safe for use in these patients.

The encouraging results with olcegepant prompted development of several orally-active gepants that were selected for clinical trials. Telcagepant, MK-3207 and BI 44370 all showed significant reduction of headache pain after two hours (Fig. 2B) [46,47]. In addition, the gepants significantly reduced photophobia, phonophobia, and functional disability at 2 hours and sustained freedom from pain after 24 hours. Testing of these compounds proceeded into Phase II and III clinical trials that demonstrated efficacy in both acute and preventive treatment of migraine symptoms (Timeline, Box1) [10].

Unfortunately, the positive momentum to develop a gepant for clinical use in migraine came to a halt over the concern of liver toxicity [10]. The first suggestion of this issue arose during a 2009 trial of oral telcagepant for migraine prophylaxis when some patients, who had been taking the medication twice daily for 12 weeks, exhibited elevated levels of liver enzymes [10]. Within a few years, clinical development of all of the initial gepants (telcagepant, MK-3207, BI 44370 TA, BMS-927711) had been discontinued.[5,49].

Nevertheless, due to the effectiveness of CGRP antagonists in aborting migraine attacks, there is renewed interest in developing gepants that are devoid of liver toxicity. Currently, ubrogepant (MK-1602) and atogepant (MK-8031), two gepants that belong to a different chemical series than telcagepant and the other gepants, are undergoing clinical testing [10]. Ubrogepant has completed Phase IIb testing and is being evaluated in Phase III trials for acute

relief of migraine. To date, it has demonstrated good efficacy, comparable to triptans, with no incidence of elevated liver enzymes or other serious adverse effects [10,48]. If the newer gepants prove to be safe as well as effective, they will provide a useful alternative to triptans, particularly for migraine patients with cardiovascular risk factors, triptan non-responders, and patients with triptan-induced medication overuse headaches [5].

Prophylactic Efficacy of Antibodies to CGRP and CGRP Receptors in Migraine

An alternative strategy for blocking CGRP transmission in migraine patients is to use selective monoclonal antibodies that bind either CGRP or the CGRP receptor. This approach has been successful for decreasing the number of migraine days in episodic and chronic migraine patients (Fig. 2C). Four such antibodies have now completed Phase III clinical trials in anticipation of submission to regulatory agencies for marketing approval (Table 1). Currently, the CGRP receptor antibody erenumab is under evaluation by FDA and EMA; and if successful, it is anticipated the drug will be marketed in 2018.

The therapeutic goal for migraine prophylaxis is to reduce the number of migraine days experienced by patients who suffer frequent attacks. Antibodies to either CGRP or the CGRP receptor have demonstrated efficacy in reducing migraine days in patients with episodic (1 – 14 days/month or fewer) or chronic ($\geq$15 days/month) migraine headache (Table 1) [10]. The anti-receptor antibody erenumab (AMG334) is a human IgG2 monoclonal antibody targeted to the CGRP binding site on the CGRP receptor. It is administered once a month by subcutaneous injection to effectively reduce migraine frequency [60-62]. Currently, there are three different monoclonal antibodies targeted to sites on the CGRP peptide that have completed Phase III clinical trials; all show positive effects for migraine prevention (reported to the 18th International

Headache Congress, Vancouver, Canada, September 2017). Overall, the anti-CGRP antibodies appear to show similar efficacy and safety profiles [10]. Eptinezumab (ALD403) is a genetically-engineered humanized IgG1 monoclonal antibody that is formulated for intravenous administration and intended for once per quarter dosing [63]. Fremanezumab (TEV-48125) is a fully humanized IgG2a monoclonal antibody that is given once per month by subcutaneous injection [64]. Galcanezumab (LY2951742) is a fully humanized IgG4 monoclonal antibody that also is administered subcutaneously on a monthly basis to reduce the number of migraine days [65]. In addition, galcanezumab has been shown to be effective in treating cluster headache , another disorder in which CGRP is released during the headache phase [54].

The antibodies are particularly suited for use as a prophylactic treatment for migraine with advantages of patient adherence and tolerability. They have a prolonged serum half-life (20-50 days) that enables patients to take their medication less frequently in order to prevent migraine attacks. Antibodies bind their target site with high affinity and selectivity, thus reducing the potential for unwanted, off-target effects. In contrast to small exogenous molecules such as the gepants, antibodies are not processed by the liver, thus avoiding the potential for liver toxicities and hepatic drug interactions. No adverse cardiovascular or cerebrovascular effects have been reported so far for the antibodies under development [9,16,17,66,67]. A primary disadvantage of antibodies, however, is they are not orally active and must be administered by injection. Injection-site reactions, including pain, are a commonly reported adverse event [5,16,67]. These reactions are usually mild and transient and are less likely with intravenous as compared to subcutaneous administration. This effect, as well as other adverse effects reported during the trials, however, was not different between the antibody and placebo-treated patients [5,66]. Thus

overall, the anti-migraine antibodies have been shown in clinical trials to be effective, well-tolerated and safe in patients.

Potential Risks of Long-Term CGRP Blockade

In spite of the promising data from recent clinical trials of CGRP and CGRP-receptor antibodies, it must be acknowledged that potential risks of long-term blockade of CGRP signaling are not known [68]. There is still much to be learned about the physiological and pathophysiological roles of CGRP, but clearly this peptide has effects throughout the body[12]. Circulating antibodies could affect all peripherally accessible sites where CGRP acts.

In particular, CGRP is a very potent vasodilator throughout all vascular regions [12,69]. What might be the impact of a chronic reduction of CGRP effectiveness on cardiovascular pathophysiology such as hypertension, cardiac dysfunction, and episodes of coronary or cerebral ischemia [68,70,71]? So far, no cardiovascular adverse effects of the CGRP and CGRP receptor antibodies have been reported with up to 6 months of Phase III clinical testing [5,16,17,66]. No patients developed hypertension related to the treatment.

The subjects in the antibody trials so far are mainly of middle age and are not a high-risk population for stroke or acute myocardial infarction. Elderly patients, in whom ischemic events are of particular concern, are less likely to experience migraine and would likely not need the antibody medications. In a recent study, erenumab was found to have no effect on patients with angina pectoris (males, about 65 yrs of age) who were monitored by EKG while exercising on a treadmill until they reported chest pain [72].

Because the antibodies will decrease, but not entirely eliminate CGRP signaling, their effect may be more significant in migrainers where the levels of CGRP release are elevated. It is

possible that compensatory mechanisms are triggered during the course of chronic treatment. As with any new class of drugs, it will be important to continue to monitor various patient populations for possible risks associated with long-term treatment of CGRP-related antibodies.

Role of CGRP in the trigeminovascular system

To better understand how CGRP-targeted therapies affect migraine, it is important to define the role of CGRP in the trigeminovascular system, a key component of the pain pathway for headache (Fig. 3A) [9]. As discussed below, there has been good progress in elucidating the trigeminovascular locations of CGRP and its receptors and major functions of this neuropeptide. There is still much to be learned, but these data, along with recent clinical studies, suggest that long-standing hypotheses regarding mechanisms of migraine headache need to be re-evaluated. The new findings contribute to an updated view of migraine pathology and sites of action for anti-CGRP therapeutic drugs.

Expression of CGRP and CGRP Receptors in the Trigeminal Ganglion

The sizeable population of CGRP neurons within the ganglion signifies a major role for CGRP in trigeminal transmission (Fig. 3B). About half of all neurons in the trigeminal ganglion express CGRP, as visualized using immunohistochemical staining with CGRP antibodies [73-75] and *in situ* hybridization to localize mRNA for CGRP [76]. CGRP-positive nerve cells are predominately of small-medium diameter, which is indicative of cell bodies of C-type sensory pain fibers. Trigeminal CGRP neurons are heterogeneous in that certain subpopulations exhibit co-localization with various other transmitters, e.g., substance P or PACAP (pituitary adenylate cyclase-activating polypeptide) [14,77], and ion channels, e.g., TRPV1 cation channel [78]. The full

extent and implications of these co-expressions have yet to be determined. The majority of CGRP neurons in the human trigeminal ganglion also express 5-HT_{1D} (90%) and 5-HT_{1B} (65%) receptors, indicating a relevant target for triptan drugs [79]. Interestingly, co-localization of CGRP with the CGRP receptor is rarely observed [73], implying a lack of autoreceptors on CGRP neurons. Within the ganglion, CGRP also is found in thin nerve fibers proximal to the cell bodies where the neuropeptide co-localized with the synaptic vesicle marker SNAP-25 [73,80]. The beaded structure of these fibers suggests they are CGRP release sites on local branches of CGRP axons (Fig. 3B).

The receptors for CGRP are found in about a third of the ganglion neurons, but these cells are distinct from those that contain CGRP (Fig. 3B). Receptor-expressing cells have been determined by co-localizing immunoreactivity for CLR and RAMP1 [73,75] or by visualizing an antibody targeted to the ligand-binding site spanning the CLR and RAMP1 subunits [74]. CGRP receptors are found in the larger neurons and thicker fibers that correspond to Aδ sensory neurons.

Interestingly, certain glial cells within the ganglion also express CGRP receptors. In particular, the satellite glial cells that surround neuronal cell bodies are labeled using either an antibody to the CGRP receptor binding site [74] or by co-localization of CLR and RAMP1 antibodies [73,75,81]. Satellite glia that contain CGRP receptors are often organized around a CGRP-expressing neuron (Fig. 3B), which is suggestive of neuronal-glia communication. The significance of this type of signaling is not well understood, but CGRP has been shown to activate the release of inflammatory cytokines and nitric oxide from ganglionic glial cells [81-85]. These substances in turn can enhance CGRP release, thus creating a positive feedback loop within the ganglion .

Overall, the relationship of cells expressing CGRP and CGRP receptors in the trigeminal ganglion (Fig. 3B) is consistent with local CGRP signaling. C-type sensory neurons may release CGRP from either the soma or local axon varicosities [87]. CGRP could then act on receptors located on Aδ-type sensory neurons and satellite glial cells to modulate pain sensitivity and transmission within the ganglion. These actions of CGRP are likely involved in migraine pathophysiology and are targets of anti-CGPR therapies.

Expression of CGRP and CGRP Receptors in Trigeminal Nerves

In bipolar trigeminal sensory neurons, CGRP is released at both peripheral and central nerve terminals. CGRP and CGRP receptors have been visualized in both the peripheral and central branches of the trigeminal nerves [73]. However, these two components are localized in distinct fibers, consistent with the expression pattern observed in ganglion cell bodies (Fig. 3B)[80]. CGRP immunoreactivity is found in the small diameter, unmyelinated C-fibers that are known to slowly transmit signals of dull, diffuse pain. In contrast, CGRP receptors are observed in thicker, myelinated fibers indicative of Aδ-type nociceptive nerves that signal sharp, acute pain [80]. The precise location in these fibers is not entirely clear in that receptor immunoreactivity has been reported for axons [73,80] and/or some of the Schwann cells surrounding the axons (co-localized with myelin basic protein) [74,75]. The close association of C and Aδ fibers within the trigeminal nerves suggests possible CGRP signaling between the two types of pain fibers and possible neuron-glia communication (FIG. 3B) [80].

CGRP and CGRP Receptors in Trigeminal Peripheral Targets

Cerebrovasculature

Shortly after the discovery of CGRP, Edvinsson and colleagues reported the presence of this peptide in nerves innervating cerebral blood vessels and demonstrated that CGRP potently dilates cerebral arteries [14,35]. Moreover, the perivascular CGRP nerves were shown to be sensory fibers originating in the trigeminal ganglion [14]. For over 30 years since these discoveries, the cerebrovasculature has provided scientists with an important window into trigeminal sensory function and a useful experimental tool for elucidating CGRP mechanisms and CGRP receptor pharmacology [11].

Trigeminal CGRP nerves are located within the vessel wall at the adventitial-medial border, and they release CGRP from axonal varicosities that are separated from the adjacent smooth muscle by a relatively wide cleft (100-500 nm) [52,88]. CGRP acts on cerebrovascular smooth muscle cells to increase cAMP, decrease intracellular Ca^{2+} and cause vasorelaxation, but it has no effect on cerebrovascular endothelium [35,89]. CGRP-mediated dilation of cerebral arteries is inhibited by CGRP receptor antagonists (gepants) as well as by antibodies targeted to the CGRP receptor [89-92].

The cerebrovascular trigeminal nerves have important effects for maintaining blood flow to the brain. CGRP increases cerebral blood flow by potently dilating cerebral arterioles, but not cerebral veins [93,94]. CGRP innervation plays a critical role in protecting the brain circulation by counteracting cerebral artery constriction (Fig. 3C). This was demonstrated by denervating the trigeminal input, which did not alter resting cerebral blood flow, but did prolong vasoconstrictor responses of cerebral arteries exposed to a variety of stimuli *in vivo* [93]. The trigeminovascular vasodilatory reflex in response to vasoconstriction is characteristic of the role sensory nerves play throughout the body to trigger protective reflexes to harmful stimuli.

It has been postulated that anti-migraine drugs work by inhibiting CGRP-mediated dilation of cerebral arteries, which are located on the pial surface of the brain. However, the endothelium in these vessels form a barrier that restricts passage of molecules from the vessel lumen to the outer layers of the vascular wall that contain the smooth muscle and CGRP nerve endings. This concept was demonstrated in isolated rat middle cerebral arteries that were cannulated and luminally perfused, allowing application of drugs to either endothelium exposed in the lumen or to adventia/smooth muscle layers on the abluminal side of the artery. In these experiments, CGRP was only effective when applied to the abluminal, but not the luminal, surface of the vessel (Fig. 3C) [89,91,95]. Moreover, neither CGRP receptor antagonists (olcegepant, telcagepant) nor CGRP antibodies were effective when given in the lumen; they only blocked CGRP-mediated dilation from the abluminal side [89,91]. Thus, CGRP receptor antagonists and circulating antibodies to CGRP and the CGRP receptor, that are effective in migraine, do not appear to be able to access potential targets in the cerebrovasculature.

Cranial Dura Mater

Trigeminal nerves project to the dura mater where they are involved in meningeal nociception and vasodilation [96,97]. Within the dura, CGRP immunoreactivity is observed in unmyelinated C-fibers, whereas the receptor components CLR and RAMP1 are co-localized in myelinated A-fibers [80,98]. The role of CGRP receptors in A-fibers is not known, but activation by CGRP could facilitate cross-talk between the two types of sensory nerves to perhaps amplify or sensitize nociceptive signaling.

CGRP nerves innervate the dural vasculature, including the middle meningeal artery and superior sagittal sinus [80,99,100]. Similar to pial cerebral vessels, CGRP receptor proteins are

expressed in the smooth muscle, and CGRP acts here to cause vasodilation [80,100]. However, there is no endothelial barrier in these vessels. Interestingly CGRP receptors were not found in dural mast cells of humans, although this has been reported for the rat [80].

Unmyelinated CGRP fibers also terminate in non-vascular regions of the dura where they are activated by noxious and harmful stimuli [80,97]. The fibers transmit pain messages to the trigeminal ganglion; and in addition, antidromic axon reflexes induce release of CGRP within the dura to increase blood flow to the region. Other neuropeptides are released as well, and together they mediate the process of neurogenic inflammation [80,101].

Direct activation of trigeminal afferents in the dura causes a painful headache in humans, as first demonstrated in 1940 by Ray and Wolff, who stimulated these nerves during surgical operations [102]. Similarities with migraine headache led to the vascular theory of migraine that postulated headaches in this disorder were triggered by dilation of intracranial blood vessels [103]. However, this view is now being discounted, particularly in light of recent studies showing that cerebral and extracranial meningeal arteries are not dilated during a migraine attack [104] nor do they cause headache when they are dilated [105].

A second theory of migraine was based on the theory that neurogenic inflammation in the dura is the trigger for migraine attacks [106]. However, numerous drugs that block the plasma protein extravasation component of neurogenic inflammation in the dura of animals have failed to demonstrate anti-migraine efficacy in clinical trials [107]. Moreover, CGRP does not induce neurogenic inflammation in humans or rodents but only mediates the vasodilatory aspect of inflammation [107]. Thus, the dura does not appear to be the primary site where CGRP-targeted therapies act to relieve migraine headache. The current view discounts dural mechanisms as the migraine trigger and instead focuses on neural origins [108,109]. Nevertheless, there is no access-

barrier within the dura or its vessels to exclude the CGRP-related drugs, so it is possible that actions here may contribute to the overall therapeutic effect.

CGRP and CGRP Receptors in Trigeminal Central Targets

Spinal Trigeminal Nucleus and Spinal Cord

CGRP is abundant in the central terminals of primary trigeminal afferents. CGRP-positive axons enter the brain and terminate in the spinal trigeminal nucleus (STN) of the caudal part of the brainstem and in the upper cervical levels of the spinal cord (C1 and C2), notably in laminae I and II of the dorsal horn [110-114]. At these central sites, trigeminal input is relayed to second-order neurons of the pain pathway that project via the brainstem and midbrain to higher cortical pain regions [108,115,116].

Similar to the peripheral branches, the central CGRP-immunoreactive fibers are thin, unmyelinated axons that display "pearl-like" structures. CGRP co-labels with synaptic vesicle protein, which indicates the presence of vesicular release sites [110]. These fibers are primarily derived from the ipsilateral trigeminal ganglion. CGRP receptor components CLR and RAMP1 also are observed, but they are found in thicker fibers that express a marker for Aδ-fibers [110].

In human STN, the highest density of CGRP immunoreactive fibers is observed in a network around fiber bundles in the superficial laminae [110]. In the cervical spinal cord, CGRP labeling is most intense in laminae I and II, and weaker in lamina V [110]. Interestingly, no neurons in these regions express CGRP or CGRP receptors; instead these elements are only observed in nerve terminals. This suggests CGRP may act to modify the signaling of Aδ-fiber afferents.

CGRP and CGRP Receptors in Ascending Pain Pathways and other CNS sites

The location of CGRP and CGRP receptors has been extensively mapped throughout the central nervous system [117-119]. These elements are found in numerous sites associated with pain processing and other functions associated with migraine symptoms.

However, because of the blood-brain barrier (BBB) in these sites, it is unlikely they are targets for therapeutic actions of CGRP-related antibodies. Detailed studies on the BBB that used Evans blue-albumin permeability and the quantitative permeability surface area method of Fenstermacher, demonstrated that there was >30 times more passage in the trigeminal ganglion as compared to the CNS and the trigeminal nucleus caudalis [77,120]. The limited passage of the antibodies (<0.01%) and some gepants (2%) into the brain speaks against the CNS as a primary site for their therapeutic action [9]. From a pharmacological point of view, the amount of drug necessary to inhibit CGRP signaling in the CNS cannot be achieved, and hence the main target must be outside the BBB. Several CNS sites that lack a BBB express CGRP and/or CGRP receptors. For example, the area postrema [118], which may play a role in migraine-associated symptoms such as nausea and vomiting, would be accessible to CGRP-related drugs. The trigeminal nerves have other projections outside the BBB that express CGRP and its receptors (Fig. 3A), such as the sphenopalatine ganglion which facilitates cross-talk between the sensory and parasympathetic systems.

An Updated View of Migraine Attacks and Therapeutic Targets

The recent findings regarding CGRP and the effectiveness of CGRP-targeted therapies in migraine offer new insight into the pathophysiology of migraine headache. However, questions remain as to how CGRP mechanisms fit in the overall theory of migraine. While there are many causes of headache, migraine is a primary neurological disorder with recurrent attacks that

appear to originate in the brain. A number of CNS areas are active during the prodrome, aura, and headache phases of migraine [108,109,121]. Interestingly, although sumatriptan relieves migraine headache, it does not inhibit ongoing activation of brainstem areas that were referred to as "migraine generators" [109,122]. It is striking that a number of migraine therapies, e.g., certain triptans, gepants and the CGRP-related antibodies, have little or no ability to cross the BBB; and thus they appear to act outside the CNS [91,123].

A peripheral site of action for CGRP-related therapies fits with traditional theories of migraine that postulated headache was triggered by either vasodilation of cranial arteries or by neurogenic inflammation in the dura. However, these theories have not held up against more recent observations [104,107,124], indicating another peripheral site must be involved in generating/maintaining the headache phase of migraine.

It is apparent that the trigeminovascular pathway is activated during a migraine attack, and the brain interprets signaling in this pathway as headache pain, as Wolff dramatically demonstrated in his surgical patients [102]. The CGRP results also clearly indicate an important role for peripheral aspects of the trigeminal system during the headache phase. CGRP is released into the cranial circulation during an attack, most likely from neurons and fibers of the trigeminal ganglion, where 50% of the neurons contain CGRP and there are no barriers to the peripheral circulation. Normalization of CGRP levels in samples from the jugular vein corresponds to resolution of the headache by triptans. This indicates trigeminal CGRP release is a good indicator of the attack, but what exactly is its role in headache generation? Because the trigeminal ganglion is central to the trigeminovascular pain pathway, it is tempting to speculate that it is the target of the new therapies and that blocking CGRP transmission within the trigeminal ganglion is sufficient to abort or prevent the debilitating symptoms of migraine.

Trigeminal Theory of Migraine Headache

Clearly, migraine is a complex, multi-symptom disorder that involves altered function at numerous peripheral and central sites. The most debilitating aspect of migraine, however, is the painful headache phase. Based on what we have learned about the CGRP system and the effectiveness of new CGRP-related drugs, it is hypothesized that the painful, lasting headache may be a consequence of dysfunctional signaling within the trigeminal ganglion. To stimulate discussion going forward, we propose a migraine model with this viewpoint (Fig. 4). It is hypothesized that during the headache phase, persistent activation of the trigeminal ganglion and its local CGRP circuits could amplify the strength and duration of signaling in central trigeminal afferents that convey the sensation of head pain to the CNS. Thus, the ganglion may act as an amplifier promoting intense and long-lasting pain. Precisely how the ganglion is initially activated during migraine still needs to be determined. The most likely targets of therapeutic CGRP antibodies and CGRP receptor blockers may be within the ganglion itself to abort and/or prevent amplification of the pain signals. While much remains to be learned about how CGRP transmission in the trigeminal ganglion is stimulated in migraine, development of effective drugs that act on this target is a significant milestone for migraine patients seeking headache relief.

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Figure Legends

Figure 1. Components of CGRP transmission and sites of action for CGRP-related migraine therapies. **A)** Amino acid structure of human αCGRP. **B)** Schematic drawing of the CGRP receptor complex that consists of two integral membrane proteins, CLR (calcitonin receptor-like receptor) and RAMP1 (receptor activity-modifying protein, type 1), and two cytoplasmic-associated proteins, RCP (receptor coupling protein) and Gαs (α subunit of the G_s protein). **C)** The targets for CGRP-related migraine therapies are illustrated using the example of a CGRP-containing trigeminal nerve varicosity innervating a cerebrovascular smooth muscle cell.
Abbreviations: AC: adenylate cyclase, PKA: protein kinase A

Figure 2. Clinical data that substantiate the significant role of CGRP in migraine headache and its treatment. **A)** CGRP release during headache. Blood samples were taken from the external jugular vein of migraineurs during either a headache attack or a headache-free period (control) and analyzed for CGRP[53]. Table inset: Samples taken during the headache phase of migraine with and without aura and following successful treatment with sumatriptan. Also samples from patients experiencing either cluster headache or chronic paroxysmal headache. CGRP, vasoactive intestinal peptide (VIP), substance P (Sub P) and neuropeptide Y (NPY) were measured [21,23,53,54,125,126]. ↑ Significant increase over control; ▬ normal blood level. **B)** Efficacy of gepants (CGRP receptor antagonists) in treating acute migraine headache. Patients with moderate-to-severe migraine headache were injected i.v. with olcegepant or given telcagepant orally and compared with placebo. The graph shows the % of patients with headache relief after 2 hours; relief was defined as a reduction of symptoms to the level of mild or no headache [45,47]. **C)** Efficacy of CGRP-related monoclonal antibodies to reduce the frequency of attacks in

patients with episodic migraine. The graph shows the percentage of patients who had >50% reduction in the number of migraine days/month after chronic treatment with either the active drug or placebo. Data are summarized from published results of Phase II clinical trials for the anti-CGRP receptor antibody erenumab and anti-CGRP antibodies eptinezumab, galcanezumab and fremanezumab [62,63,65,67].

Figure 3. CGRP and CGRP receptors in the trigeminovascular system. **A)** Illustration of the trigeminovascular pathway and CNS sites where CGRP and/or CGRP receptors have been localized using immunohistochemistry. The receptor expression pattern resembles that of CGRP, however CGRP does not co-localize with receptor elements in either cell bodies or fibers. One exception is rat cerebellar Purkinje cells where CGRP is found in the cytoplasm and the receptor elements are present on the cell surface. *Abbreviations: FN – facial nucleus, IV - 4th ventricle, LC - locus coeruleus, MRN - magnus raphe nucleus, PAG – periaqueductal gray, PC – Purkinje cells, SPG – sphenopalatine ganglion, SSN - superior salivatory nucleus, STN - subthalamic nucleus, TG – trigeminal ganglion, TNC - trigeminal nucleus caudalis.* **B)** Schematic drawing showing the relationship of cells and fibers within the trigeminal ganglion that express either CGRP or the CGRP receptor, as discussed in the text. **C)** CGRP regulation of cerebral arteries located on the pial surface of the brain. *Top:* CGRP is a potent dilator when applied to the abluminal side of isolated arteries but has no effect when its application is restricted to the vessel lumen [91,95]. *Bottom:* CGRP mediates the trigeminovascular reflex that protects the cerebral circulation from excessive vasoconstriction. Its role is demonstrated by comparing the effect of various vasoconstrictors on cerebral arteries *in vivo* when the trigeminal innervation is either intact or lesioned. [93].

Figure 4. Trigeminal theory of migraine headache and relief by CGRP-targeted therapies. The migraine attack is initiated in the CNS, likely involving regions in the dorsal pons, hypothalamus and thalamus that are referred to as "migraine generators" [109]. During the headache phase, the trigeminal ganglion (TG) is activated, triggering stimulation of centrally-projecting afferents of the trigeminovascular pathway that convey the sensation of head pain to the CNS. These afferents terminate behind the blood-brain barrier in the spinal trigeminal nucleus (STN) and the dorsal horn of spinal levels C1 and C2. Second-order neurons then project the pain signal through ascending pain pathways to thalamic and cortical regions that perceive headache pain. The TG is proposed to act as a "migraine amplifier" of headache pain due to persistent activation of CGRP circuits within the TG that reverberate via cross-talk among sensory neurons (C-fiber and Aδ-fiber) and satellite glia (Fig. 3B). Blocking CGRP signaling within the TG would abort the migraine headache and/or prevent it from occurring. New CGRP-targeted therapies, i.e., CGRP antibodies, the CGRP receptor antibody and gepant receptor antagonists, can all access the barrier-free TG to exert this action.

BOX 1: Timeline

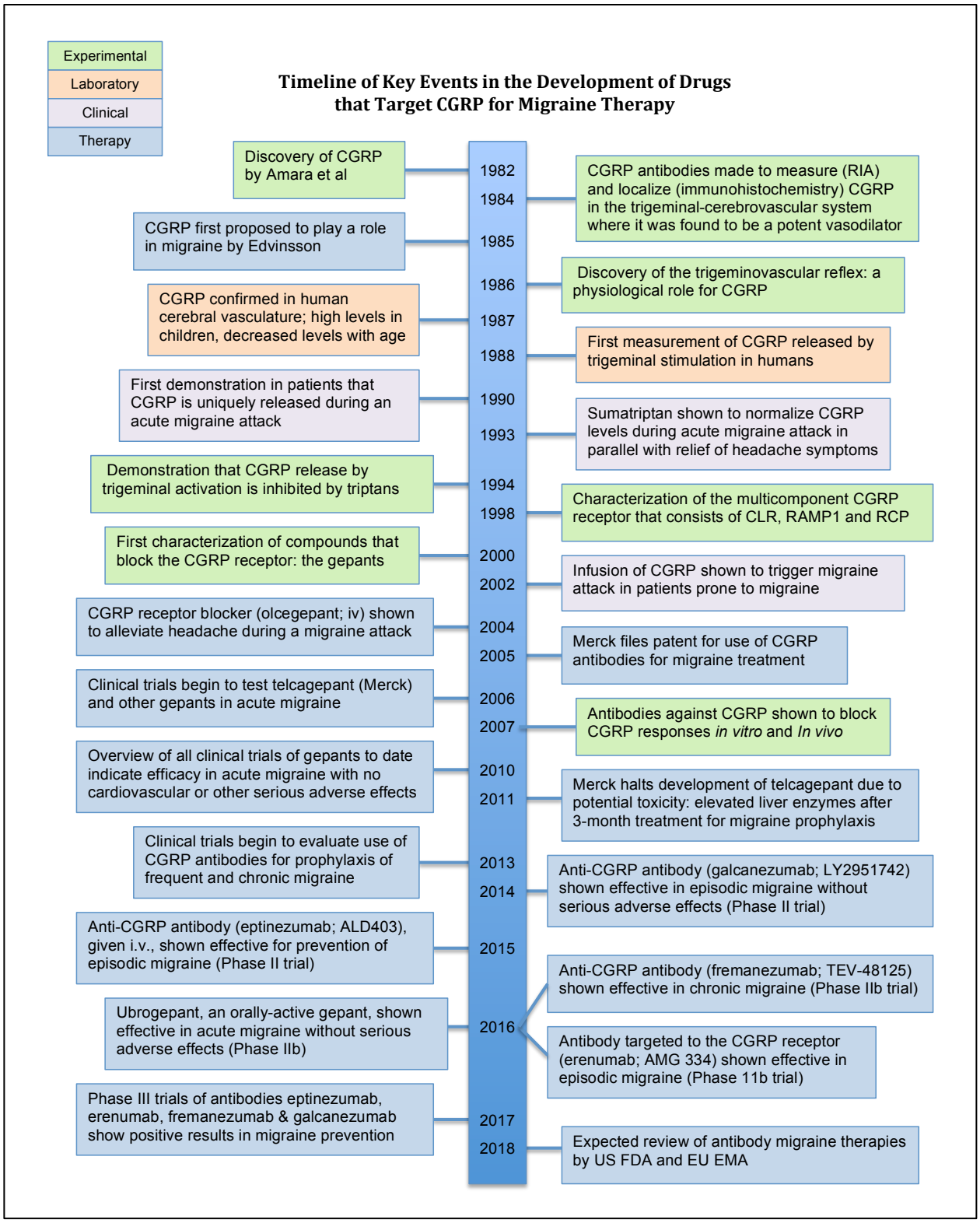


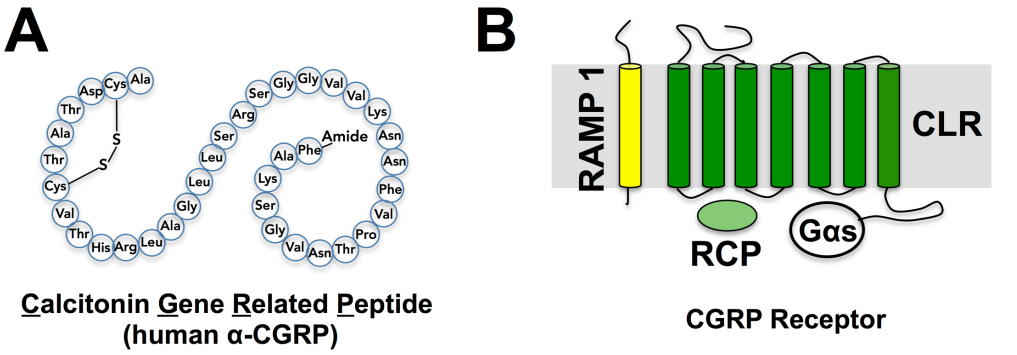
Table 1: CGRP-related therapies for migraine and other headache disorders

Drug	Indication[1]	Dosing	Mechanism	Drug Development Status (Sep. 2017)
Preventive Therapy				
Erenumab (AMG 334)	Migraine prevention in episodic (EM) & chronic migraine (CM)	Monthly, SC	Monoclonal antibody to CGRP Receptor	Completion of Phase III trials; registration study published; submitted for US FDA & EMEA review
Galcanezumab (LY2951742)	Prevention of migraine (EM, CM) & cluster headache (eCH, cCH)	Monthly, SC	Monoclonal anti-CGRP antibody	Positive results reported[2] for Phase III trials in EM and CM
Fremanezumab (TEV-48125)	Prevention of migraine (EM, CM) & cluster headache (eCH, cCH)	Monthly or Q3, SC IV load for CH	Monoclonal anti-CGRP antibody	Positive results reported[2] for Phase III trials in EM & CM
Eptinezumab (ALD403)	Prevention of migraine (EM, CM)	Q3, IV	Monoclonal anti-CGRP antibody	Positive results reported[2] for Phase III trials in EM; Phase III for CM ongoing
Acute Therapy				
Ubrogepant	Relief from acute migraine attack	Oral, as needed	CGRP receptor antagonist	Positive Phase IIb results; Phase III ongoing

[1]Prevention defined as reduction in headache days in episodic migraine (4-14 days/month; EM), chronic migraine (≥ 15 days/month; CM), episodic cluster headache (eCH), or chronic cluster headache (cCH)

[2] Reported at the 18th Congress of the International Headache Society, Vancouver, Canada, September 7-10, 2017

Figure 1


A
Asp Cys Ala
Thr
Ala
Thr
Cys
Val
Thr His Arg Leu
Ala
Gly
Leu
Leu
Ser
Arg
Ser
Leu
Ala Phe Amide
Lys
Ser
Gly Val Asn Thr Pro
Val
Phe
Asn
Asn
Lys
Val
Gly Gly Val
Ser
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S
Calcitonin Gene Related Peptide
(human α-CGRP)
B
RAMP 1
CLR
RCP
Gαs
CGRP Receptor

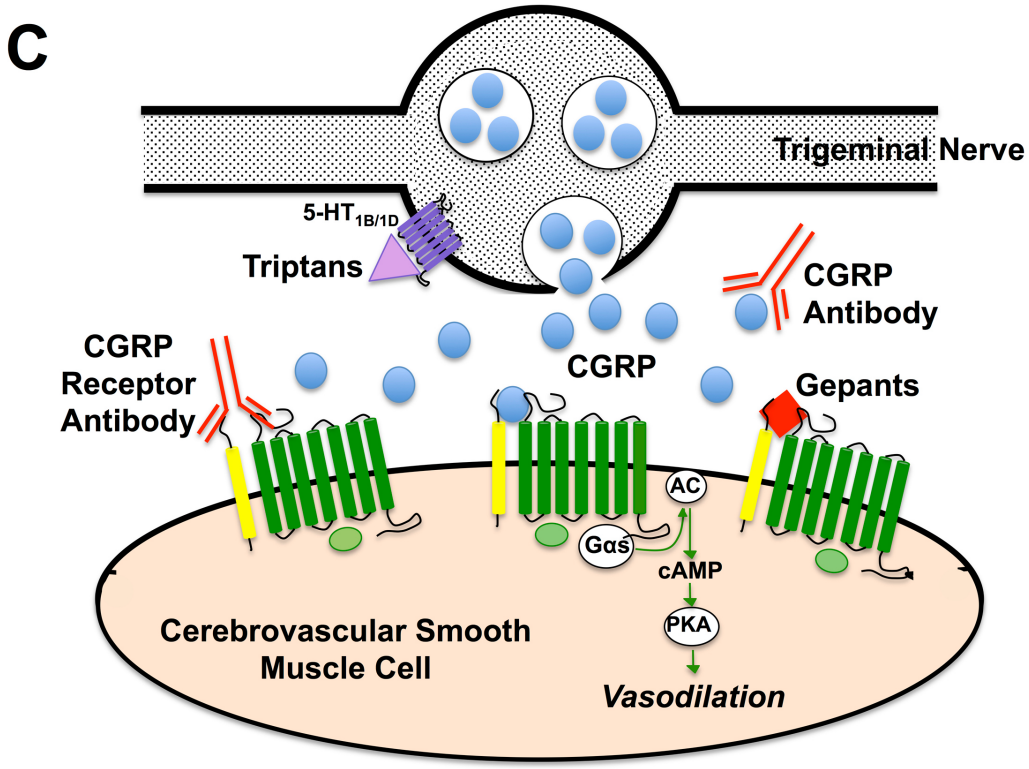
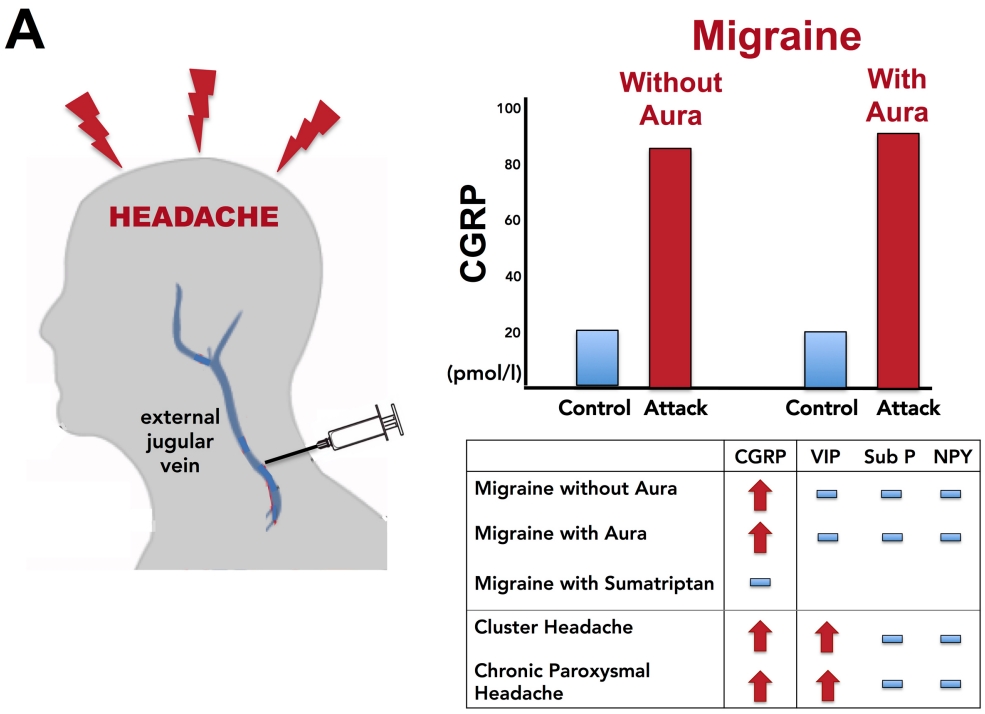

C
Trigeminal Nerve
5-HT1B/1D
Triptans
CGRP
Antibody
CGRP
Receptor
Antibody
CGRP
Gepants
AC
Gαs
cAMP
PKA
Cerebrovascular Smooth
Muscle Cell
Vasodilation

Figure 2



	CGRP	VIP	Sub P	NPY
Migraine without Aura	↑	–	–	–
Migraine with Aura	↑	–	–	–
Migraine with Sumatriptan	–			
Cluster Headache	↑	↑	–	–
Chronic Paroxysmal Headache	↑	↑	–	–

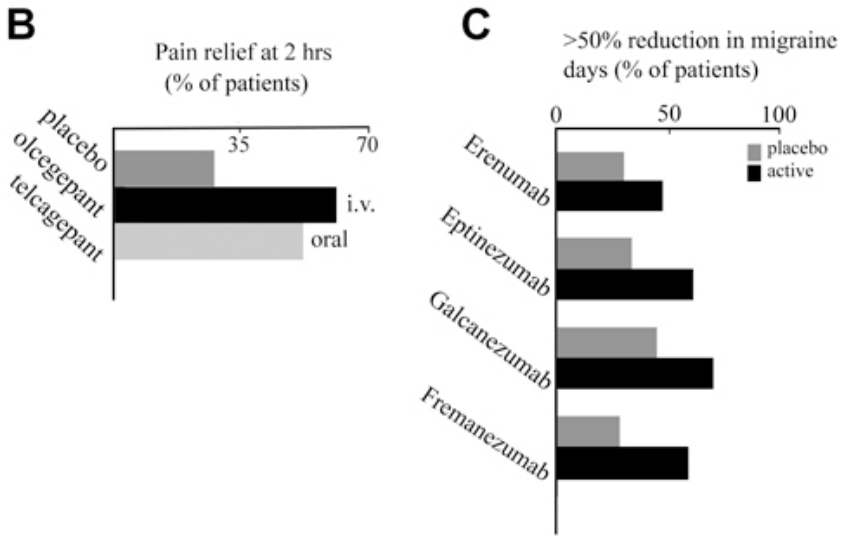
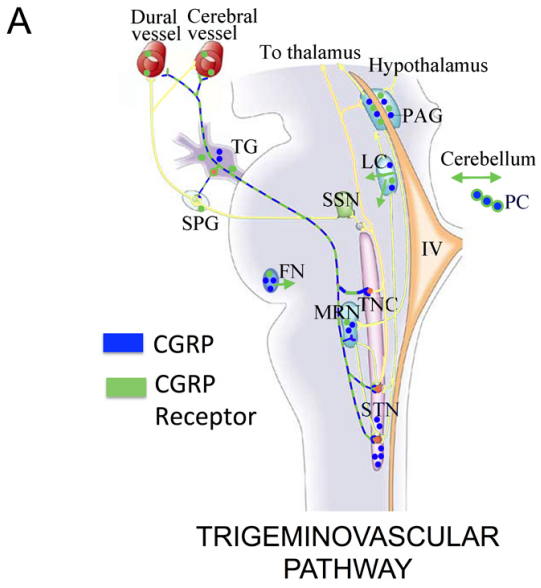
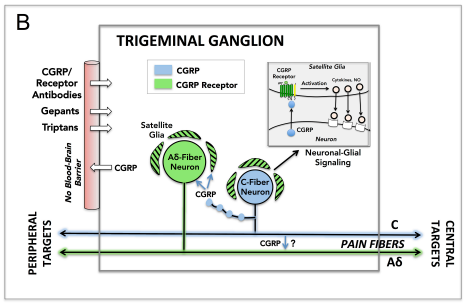


Figure 3


A
Dural vessel
Cerebral vessel
To thalamus
Hypothalamus
PAG
TG
LC
Cerebellum
PC
SSN
SPG
IV
FN
MRN
TNC
CGRP
CGRP Receptor
STN
TRIGEMINOVASCULAR PATHWAY


B
TRIGEMINAL GANGLION
CGRP/ Receptor Antibodies
Gepants
Triptans
No Blood-Brain Barrier
CGRP
CGRP Receptor
Satellite Glia
Aδ-Fiber Neuron
C-Fiber Neuron
Neuronal-Glial Signaling
PERIPHERAL TARGETS
CENTRAL TARGETS
C
Aδ
PAIN FIBERS

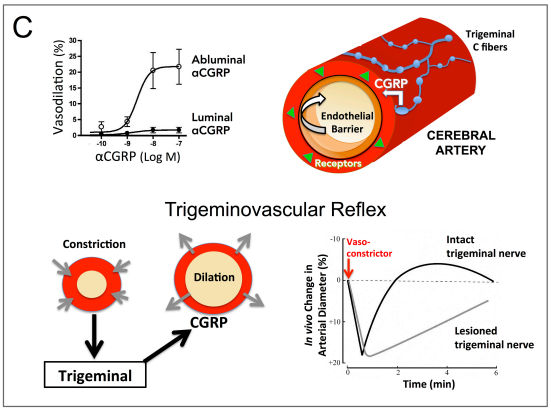
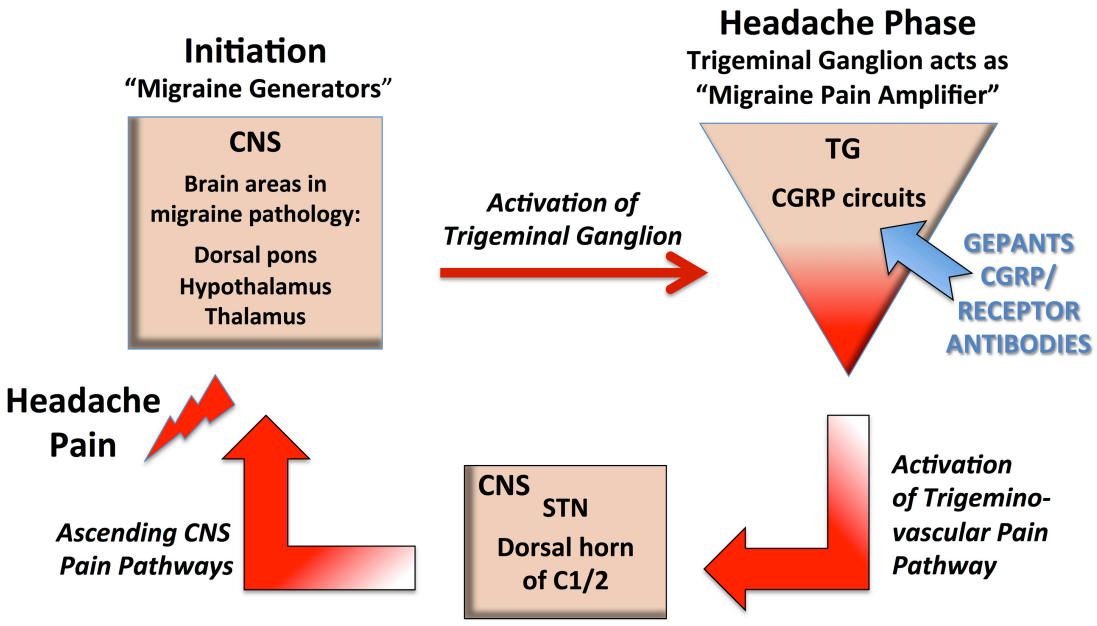

C
Vasodilation (%)
αCGRP (Log M)
Abluminal αCGRP
Luminal αCGRP
Trigeminal C fibers
CGRP
Endothelial Barrier
Receptors
CEREBRAL ARTERY
Trigeminovascular Reflex
Constriction
Dilation
CGRP
Trigeminal
In vivo Change in Arterial Diameter (%)
Vaso-constrictor
Intact trigeminal nerve
Lesioned trigeminal nerve
Time (min)

Figure 4


Initiation
"Migraine Generators"
CNS
Brain areas in migraine pathology:
Dorsal pons
Hypothalamus
Thalamus
Activation of Trigeminal Ganglion
Headache Phase
Trigeminal Ganglion acts as "Migraine Pain Amplifier"
TG
CGRP circuits
GEPANTS
CGRP/
RECEPTOR
ANTIBODIES
Activation of Trigemino-vascular Pain Pathway
CNS
STN
Dorsal horn of C1/2
Ascending CNS Pain Pathways
Headache Pain