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„ Erenumab: Long-Term Safety and Tolerability "

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Mag.pharm. Jovana Asceric

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1 Introduction

1.1 Migraine, state of the art

Since May 2018, patients with migraine – a very disabling primary headache disorder that affects around 15% of the global population, have access to an innovative group of medications that seems to be very efficient in treating as well as preventing this severe and widespread chronic health problem. Monoclonal antibodies antagonizing the CGRP pathway represent a new migraine-targeted therapy and use a unique and specific mechanism to treat migraine.

1.1.1 Types of migraine

Migraine is a common, chronic and complex neurological brain dysfunction that is classified by the International Headache Society and is published in the International Classification of Headache Disorders (ICHD). The current version of ICHD-3 beta distinguishes 6 main subtypes, most of which are further subdivided (Headache Classification Subcommitee of the IHS, 2018). Roughly migraine can be classified into two major categories:

-Migraine without aura, also known as the common migraine is a recurrent headache disorder manifesting in attacks lasting 4-72 hours. The typical characteristics are unilateral headache, pulsating pain of moderate to severe intensity, that worsens by routine activities and it is often accompanied by nausea and /or photophobia and / or phonophobia (Headache Classification Subcommitee of the IHS, 2018).

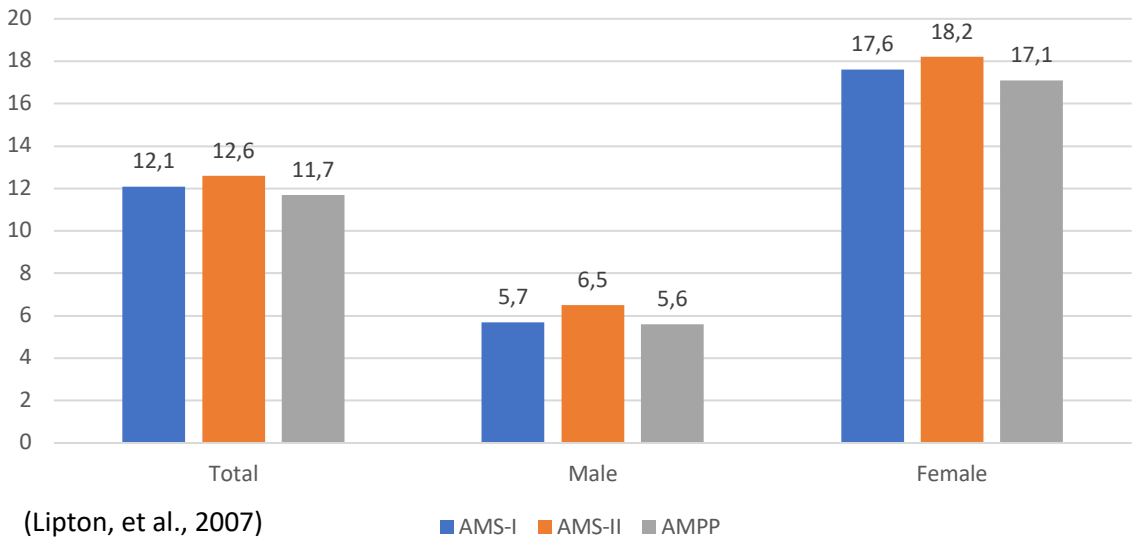
-Migraine with aura, once called classical migraine is typically characterized by reversible focal neurological symptoms (sensory, visual, speech) that precede headaches and associated migraine symptoms. Based on the aura symptoms various subtypes exist, such as the migraine with brainstem aura, this includes visual, sensory and/or speech or language symptoms and at least 2 of the following additional symptoms: slurred speech, vertigo, tinnitus, double vision, unsteadiness, and a severe sensitivity to sound. Then hemiplegic migraine is a subtype of migraine with aura that is characterized by motor weakness on one side of the body. In rare cases the attacks are accompanied with monocular visual disturbance, including scintillations, scotomata or blindness, associated with migraine headache in that case the diagnosis would be retinal (ocular) migraine. In some patients the aura is neither accompanied nor followed by headaches of any sort, this subtype is called typical aura without headache (Headache Classification Subcommitee of the IHS, 2018).

1.1.2 Prevalence of migraine

Approximately 30 million people in the United States aged from 12 to older suffer from headaches that correspond to the definition of migraine established by the International Headache Society (Lipton et al., 2001a).

In 2004 Lipton RB et al. started the American Migraine Prevalence and Prevention (AMPP) study, with the aim to reassess the prevalence of Migraine in the United States (Lipton et al., 2007). It was modeled on the methods of the American Migraine Studies I (AMS-I) (Lipton et al., 1998) and II (AMS-II) (Lipton et al., 2001a) (Lipton et al., 2001b) allowing comparisons in the findings.

Prevalence of migraine in AMS-I, AMS-II, and AMPP study for total sample and by sex.



(Lipton, et al., 2007)

FIGURE 1

In the latest study-AMPP, 162 576 individuals aged 12 years or older were assessed and 18 968 met ICHD-2 criteria for migraine. As we can see in FIGURE 1 the one year period prevalence was 11,7%, 17,1% in women and 5,6% in men, suggesting that women are more likely to be diagnosed with migraine. The results also show that the epidemologic profile of migraine remained stable in the past 15 years in the United States (Lipton et al., 2007). Latest results from a systematic review conducted in 2018 in the USA again approved that the prevalence has been stable over the years (Burch et al., 2018).

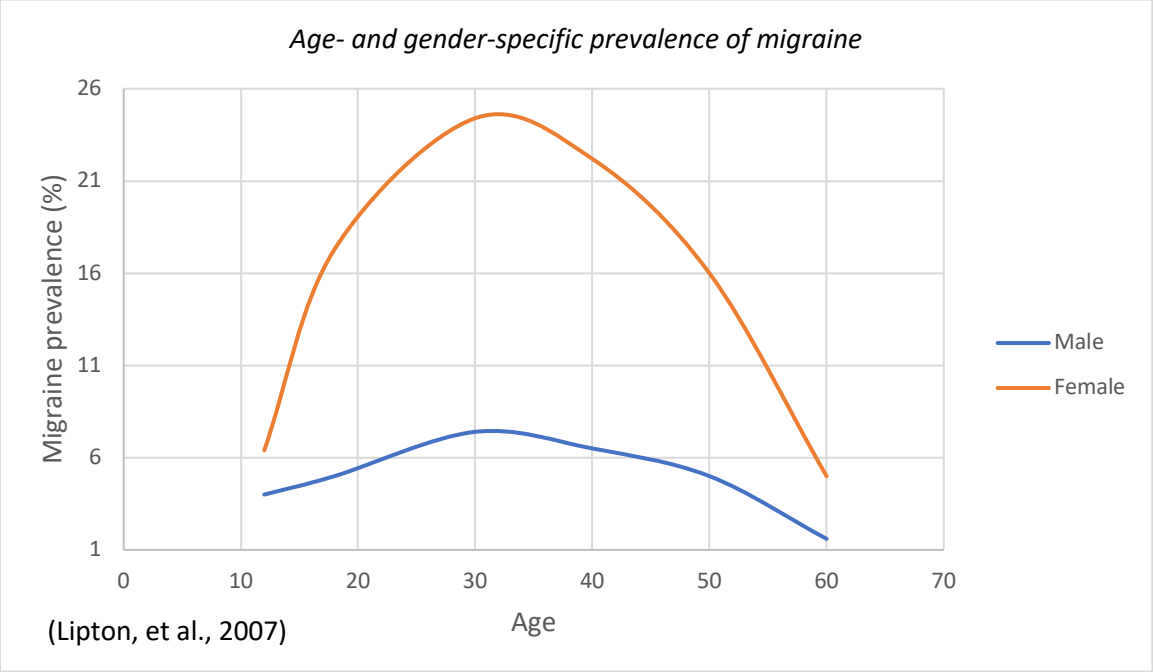


FIGURE 2

FIGURE 2 shows that in both males and females, the prevalence of migraine is an inverted U-shaped curve. The prevalence of migraine varies as a function of age: in early adult life the prevalence rises and then falls around midlife. In fact, migraine prevalence was at its highest in those aged between 30 and 39 for both women (24,4%) and men (7,4%), on the other side prevalence was lowest in those aged 60 years or older at 1.6% in men and 5.0% in women (Lipton et al., 2007). Moreover, we can again notice that migraine is more common in women than in men with a female-to-male ratio around 3:1. Interestingly at prepubertal ages it was noticed that migraine prevalence was actually a little bit higher in boys than in girls (Stewart et al., 1991).

For both men and women, the prevalence appears to be significantly higher in whites than in blacks (women 17,3% vs 13,7% and men 5,7% vs 4,1%) (Lipton et al., 2007). Finally, for both sexes, the prevalence of migraine was greater in the group of lower income (Lipton et al., 2007). Very similar results have been reported over the years in various european countries such as France (Henry et al., 1992) (Henry et al., 2002), Norway (Hagen et al., 2000) and Denmark (Russel et al., 1995)

1.1.3 Length and recurrence

The frequency of attacks is varying, from a few in a lifetime to several a week. Most migraineurs experience less than 15 migraine days or headache days per month, with or without aura and they are considered to have episodic migraine. Whereas chronic migraine is defined as at least 15 headache

days per month (of which at least 8 are migraine days with or without aura) (Headache Classification Subcommitee of the IHS, 2018).

The duration is different from type to type, in general an attack lasts from 4 to 72 hours (Headache Classification Subcommitee of the IHS, 2018). In rare and specific cases, it can last longer, these are then complications of migraine. For example, the status migrainosus and the persistent aura without infarction (Headache Classification Subcommitee of the IHS, 2018).

1.1.4 Pain locations

A study of headache location reported that the highest location frequencies were in the eyes, temporal and frontal (Kelman, 2005). In fact, migraine pain usually begins above the eyes, even though migraine can cause pain all over the head, according to various studies more than half patients reported that the pain is concentrated on one side (unilateral) with a slight 'preference' for the right side (Lipton, et al., 2001b) (Kelman, 2005). Some complained that the pain is on both sides (bilateral) or altering from one to the other side and few stated that the pain is located in the middle of the head (Kelman, 2005). According to (Kelman, 2005) the intermediate sites of pain in migraine are the occipital and the neck areas. These findings are in accordance with the results from the American Survey (2018), in which out of 4 365 respondents, 69% reported neck pain as part of a migraine symptom (Health Union). And the least common sites are diffuse and the vertex (Kelman, 2005). Overall, the results suggested that a single location is rather rare and that the locations of pain are very similar in the different types of migraine but there are some differences. The differences are mainly seen in gender, headache frequency and aura (Kelman, 2005). Finally the location shows many correlations with the stages, triggers and features of migrain (Kelman, 2005).

1.1.5 Timeline of a migraine attack

Migraine progression is different for everyone. Not every patient goes through all stages and the different symptoms can express themselves at different levels at each stage. But in general, an attack follows the same pattern. A typical migraine can have up to four distinct phases:

1. Prodrome phase

Before migraine pain hits, the patient may go through this so called 'preheadache' phase, which can begin hours or one or two days before the other symptoms of a migraine and/or the aura (Buzzi et al., 2005). About 60% of migraineurs experience prodromal symptoms, but not necessarily before every migraine attack (Lynn et al., 2004) The warning signs vary from person to person (Rossi et al., 2005)

but can include: feeling irritable, mood changes, fatigue, extra sensitivity to light or sound, nausea, insomnia, diarrhea or constipation, muscle stiffness in the neck and shoulder areas (Lynn et al., 2004). And very unique symptoms to the prodrome phase such as yawning, craving certain food and frequent urination can also occur (Lynn et al., 2004).

2. Aura phase

Various studies showed that up to one-third of people with migraine experience aura before the headache as a distinct phase in the progression of their migraine attack (Rasmussen et al., 1992) (Russell et al., 1995) (Launer et al., 1999). Just as other phases, aura doesn't necessarily precede every migraine attack. Aura symptoms appear gradually over few minutes and can last up to 60 minutes, they can be accompanied or followed by headache or even without headache following (Headache Classification Subcommittee of the IHS, 2018). The symptoms can be of sensory or motor nature and many people experiencing aura endure more than one. Visual symptoms occur in up to 99% of cases and in more than half of these they do not come with sensory or motor symptoms (Russel et al., 1996) Blurry vision, blind spots, flashing lights, geometric patterns and vision loss are symptoms that may be experienced (Russel et al., 1996). The second most common type is the sensory aura (30-40%), typically it affects only one side (unilateral) and it starts with symptoms such as numbness or tingling in the hand and progresses towards the face and tongue via arm (Russel et al., 1996). Finally, the motor aura, which is less common, is half-sided and affects the hand and arm (Russel et al., 1996). Other symptoms can be weakness, trouble in speaking or hearing, memory problems and vertigo (Russel et al., 1996).

3. Headache phase

The headache phase of a migraine attack is usually characterized by throbbing, pulsing pain on one or both sides of the head. The pain can move from one side of the head to the other during the progression of the headache, or more commonly, it may begin on one side and then gradually occupy the other side. The pain can last 4 to 72 hours and the severity varies from person to person and from attack to attack, with an intensity ranging from moderate to severe. Even every day activities can aggravate the pain. (Headache Classification Subcommitee of the IHS, 2018). Besides pain, headache phase symptoms can include nausea, insomnia, anxiety, blurred vision and sensitivity to sound, light and smell. For instance, nausea occurs in almost 90% of patients, and vomiting in about one-third. (Lipton et al., 2001a) (Lipton et al., 2001b).

4. Postdrome phase

Once the attack symptoms fade away, the migraine may not be over. The last phase, also known as the 'migraine hangover' takes place once the headache has settled (Bose et al., 2016). The postdrome phase is very common, but not every person suffers from it. It does not cause pain like the headache phase, but it can cause its own symptoms for 24-48 hours after the end of the migraine attack. Many reports a sore feeling in the area where the migraine occurred, some report trouble in concentrating for a few days after the headache passed. The persons may feel tired or weak and have body aches, gastrointestinal symptoms, dizziness, mood changes and sensitivity to light (Kelman, 2006). Even though the headache is over, people in postdrome are still experiencing a migraine attack and can benefit from avoiding triggers that provoke headache, like bright lights and strong smells.

1.1.6 Triggers

A trigger is anything that contributes to a migraine attack. So, they do not cause a migraine attack they bring it on. Most migraineurs report experiencing triggers (Pavlovic et al., 2014). Symptoms may start up to 24 hours after a trigger (Bartleson et al., 2010). There are no universal triggers, different people have different triggers, but the common ones are : environmental factors (weather), sleep problems, stress and food (Kelman, 2007)

- Lifestyle triggers: any kind of stress and change in sleep patterns (Kelman, 2007).
- Environmental triggers: strong smells, flickering lights, smoke, air pressure and means of transportation (Kelman, 2007).
- Weather-related triggers: humidity, sudden changes, summer/winter and pressure (Fux et al., 2018).
- Hormonal triggers: Changes in hormonal level may be a trigger in women. In fact, migraines are more likely to happen around menstruation (Radat, 2013). Other hormonal changes, for instance oral contraceptive use, pregnancy and menopause also play a role (Chai et al., 2014).
- Dietary aspects : Some food and drinks can cause migraine. Especially citrus fruits, alcohol, nicotine, tyramine-containing foods (bananas, chocolate, red wine and cheese). Tyramine indirectly stimulates the release of norepinephrine, resulting in vasoconstriction, which could be the reason for a migraine attack (Fux et al., 2018).

1.1.7 Pathophysiology

In the twentieth century migraine was thought to be a result of vascular dysregulation. Nowadays, activation of the trigeminovascular system, cortical spreading depression and neuronal sensitization are seen as particularly important factors in the pathophysiology of migraine (Cutrer, 2010) (Goadsby, 2009). It is believed that a migraine attack starts in the trigeminovascular system and involves dysfunction of brainstem pathways that normally modulate sensory input. The key pathway for pain is the trigeminovascular input from the meningeal vessels, via the trigeminal ganglion and synapses on second-order neurons in the trigeminocervical complex. In turn, nerve fibers involved in the localization of pain escalate from the trigeminal nucleus caudalis to the thalamus and then to the sensory cortex (Goadsby, 2009). The convergence of projections from the trigeminal nerve at the trigeminal nucleus caudalis and upper cervical nerve roots can take credit for pain in the head and neck. Sensitation and alteration of thalamo-cortical circuits can occur. Various sensory inputs and messages can be modulated via direct and indirect projection and by descending or ascending influences from the hypothalamus (Goadsby, 2009). Various genetic, hormonal and neurochemical factors work together and result in this dysregulation of cortical and brainstem excitability (Charles et al., 2010). Actually, the brain in patients with migraines is hyper excitable, with a reduced threshold for the initiation of cortical spreading depression (CSD). CSD is a self-propagating wave of cellular depolarization that expands across the cerebral cortex. The phenomenon is associated with altered connectivity and might be implicated in aura. (Charles, 2009). It is believed that cortical spreading depression can activate neurons in the trigeminal nucleus caudalis and provoke inflammatory changes (release of various neurotransmitters and neuromodulators such as CGRP) in pain-sensitive meningeal vascular structures (vascular changes including enhanced blood-brain barrier permeability), which finally leads to headache via central and peripheral reflex mechanisms (Gursoy-Ozdemir et al., 2004).

Finally, the phenomenon of sensitization, a learning process in which frequent administration of a stimulus results in continuous amplification of a response. In the case of neuronal sensitization, neurons become progressively more responsive to nociceptive and non-nociceptive stimulation, and this is thought to play a role in migraine attacks. The outcomes are: increased response, expansion of receptive fields, and development of spontaneous neuronal activity. Peripheral sensitization in the primary afferent neuron and central sensitization of higher-order neurons of the spinal cord and brain may play an important role in somatic pain (Malick et al., 2000) (Bernstein et al., 2012). In fact, many of migraine symptoms, such as exacerbation of headache by physical activity and allodynia are linked to sensitization (Bernstein et al., 2012).

1.1.8 Current therapies

Migraine treatment may be classified into two broad categories, it may be either abortive or prophylactic.

1. **Abortive treatment**, also known as acute or symptomatic treatment aims to stop the symptoms and is taken during migraine attacks. Trigger avoidance and acute symptomatic control with medication, are the two main methods in this type of treatment (Bartleson et al., 2010). Medications are more effective when they are used earlier in an attack (Bartleson et al., 2010). However, frequent use of symptomatic medications may result in the development of medication overuse headache, in which the headaches become more frequent and severe (Headache Classification Subcommitee of the IHS, 2018). Thus, prophylactic and symptomatic medications become ineffective.

- Pain relievers such as NSAIDs (acetylsalicylic acid, ibuprofen (Rabbie et al., 2010), diclofenac (Derry et al., 2013), naproxen), metamizole, paracetamol (acetaminophen) or the combination of paracetamol, acetylsalicylic acid and caffeine (Gilmore et al., 2011) are recomended for the treatment of mild to moderate migraine.
- Antiemetic medication is usually combined with other medications. Drugs, such as metoclopramid, domperidon and cyclizin can enhance the absorption of NSAIDs, triptans and others, when they are taken streightaway after the onset of the first symptoms. They increase gastric motility and may prevent migraine associated nausea (Derry et al., 2013) (Rabbie et al., 2010) (Derry et al., 2013b) .
- 5-HT (5-hydroxytriptamine receptor or serotonin receptor) is a key mediator in the pathogenesis of migraine. Thus, 5-HT_{1B} and 5-HT_{1D} receptor agonists, commonly known as 'triptans' have been reported as the most effective medication for the treatment of acute migraines (Ferrari et al., 2001) with moderate to severe pain or those who do not respond to simple analgesics (Gilmore et al., 2011). There are different galenics available, for routes like oral, iv. injection, nasal spray and oral dissolving tablets. Overall, they all appear equally efficient and with comparable side effects. However, one may respond better to a specific one (Gilmore et al., 2011). Triptan medications include sumatriptan, naratriptan, zolmitriptan, rizatriptan, frovatriptan, almotriptan and eletriptan.
- Other therapeutic possibilities include: ergotamines and dihydroergotamine, which are older medications with a broad spectrum of side effects; opioid medications, which are used only in cases when no other treatment provides relief and glucocorticoids (dexamethasone, prednisone) may be added to standard treatment to improve pain relief but fraquent use should be avoided because of the side effects (Colman et al., 2008).

2. **Preventive therapy** should be given to those who suffer from frequent or severe disabling migraine headaches. The therapy is indicated, when migraine attacks occur more than three times per month for more than 48 hours, when symptomatic treatment is unsatisfactory or when unacceptable side effects occur, in case of comorbidity and in case of medication overuse headache. All in all, preventive drugs are used to reduce frequency, duration and severity of migraine attacks (Silberstein et al., 2012) (Silberstein et al., 2008) (Silberstein, 2000) . The most comonly prescribed are beta-blockers, antidepressants and anticonvulsants (Silberstein et al., 2012) (Schürks et al., 2008).

- The effectiveness of beta blockers in migraine treatment was a chance finding in patients receiving propranolol for angina (Rabkin et al., 1966). Propranolol and timolol are FDA (Food and Drug Administration) approved for migraine prophylaxis. Other beta-blockers that are used in migraine treatment are nadolol, metoprolol, and atenolol (Silberstein, 2000).
- Calcium channel blockers, especially verapamil may be used in preventing migraines, but they seem to be weak and they are not first line choices in the United States (Silberstein et al., 2012) In some european countries flunarizin is approved for migraine prophylaxis and it appears to be effective for this indication (Karsan et al., 2018).
- Tricyclin antidepressants may be effective in preventing minraine in some patients, even without depression. Amitriptyline is the only tricyclic antidepressant proved to effectively prevent migraines, but it has not been approved by the FDA (Silberstein et al., 2012). The off-label use is though very common, despite its wide range of anticholinergic side effects (Schürks et al., 2008).
- Anticonvulsants are commonly prescibed for migraine prevention, because they have been shown to be highly effective in placebo-controlled trials, especially valproate acid (Hering et al., 1992) (Jensen et al., 1994) and topiramate (Silberstein et al., 2007). Both drugs are FDA-approved for this indication. Gabapentin has also been found to be effective in prevention of frequent migraine attacks (off-label use) (Mathew et al., 2001). And lamotrigine is an antiepileptic drug of choice for migraine with aura prophylaxis (Lampl et al., 2008).
- Other therapeutic options could be: biofeedback, which induces muscle relaxation (Vasudeva et al., 2003), evidences showed an improvement in incidence of chronic migraine for migraineurs when botox (botulinum toxin) treatment was used (Dodick et al., 2010), riboflavin (Boehnke et al., 2004), magnesium (von Luckner et al., 2018) and CoQ10 (Sandor et al., 2005) may also be effective in migraine prophylaxis.

Nevertheless, all migraine prevention drugs currently used are non-specific. These drugs were designed for other purposes rather than treatments that offer the advantage of targeting specific pathophysiological processes known to be active in migraine leading to better management of patients. Besides that, not all currently used pharmacologic approaches are effective for all patients, they may not meet patients' preferences, and they might even be contraindicated because of drug-drug interactions, which are frequently noticed, especially in patients with comorbidities such as cardiovascular and psychiatric diseases. Low efficacy and poor tolerability lead to low adherence rates this is why there is a need for new approaches.

1.2 CGRP and its receptor

1.2.1 CGRP

This peptide neurotransmitter that is mainly produced in sensory neurons exists in two isoforms. The α form of CGRP has been discovered after alternate splicing of the gene for the calcitonin gene (Amara et al., 1982). The β form of CGRP, β-CGRP differs from α-CGRP for three amino acids in humans and only one in rats (Amara et al., 1985). The main isoform, α-CGRP, is a 37-amino-acid peptide that is implicated in migraine. It is synthesized in neurons through tissue-specific splicing of mRNA transcribed from the calcitonin-CGRP gene (CALCA) located on chromosome 11 (Amara et al., 1982). Together with the homologous proteins pro-calcitonin, calcitonin, adrenomedullin, amylin and intermedin, as well as the C-terminal fragments, $CGRP_{(18-37)}$ and $CGRP_{(19-37)}$ they form the calcitonin receptor family of peptides (Born et al., 2010) (Poyner et al., 2002) These CGRP fragments have been identified in rat spinal cord and the $CGRP_{(19-37)}$ fragment happens to be an antagonist of the receptor (LaGreve's et al., 1997).

1.2.2 CGRP receptor

The CGRP receptor is a complex of several subunits, and each subunit is necessary for the ligand specificity and full function of the receptor *(FIGURE 3)*:

Schematic illustration of the CGRP receptor

complex complex

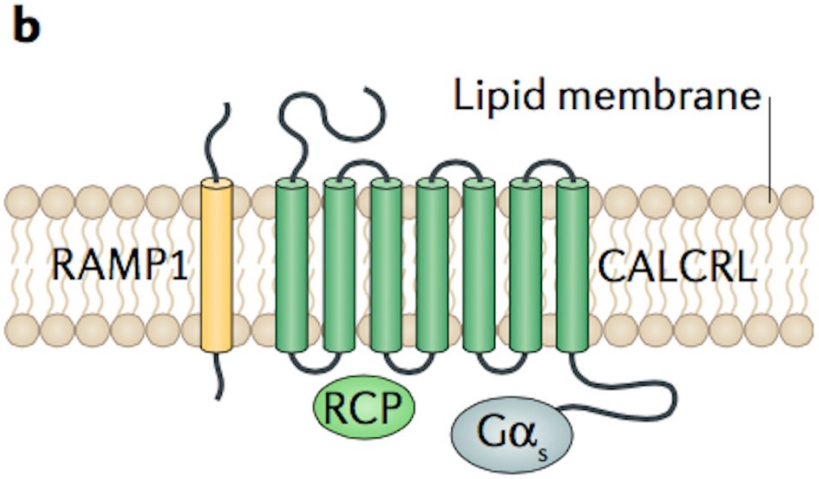


FIGURE 3

The receptor consists of two integral membrane proteins calcitonin receptor-like receptor (CALCRL or CLR) and receptor activity-modifying protein 1 (RAMP 1) and two cytoplasmic proteins, receptor coupling protein (RCP) and the α-subunit of the G protein ($G\alpha_s$). (Edvinsson et al., 2018)

1. CLR or CALCARL

Central to the complex is the 461-amino acid G-protein-coupled receptor ($G\alpha_s$) with 7 transmembrane domains-CLR (calcitonin receptor-like receptor), which is member of the secretin receptor family.

2. RAMPs

Three RAMPs (receptor activity-modifying protein) are known: RAMP1, RAMP2 and RAMP3. These are single transmembrane proteins. Only the heterodimerization of RAMP1 and CLR creates a CGRP receptor with high affinity for CGRP and that can be antagonized by the peptide $CGRP_{8-37}$, whereas the associations with RAMP2 and RAMP3 produce adrenomedullin receptors (AM1 and AM2) (McLatchie et al., 1998)

3. RCP

An additional third protein, the so-called RCP (receptor component protein) is required for an optimally functional CGRP receptor, because this hydrophilic membrane associated protein is able to activate the phosphorylation of multiple downstream targets (Evans et al., 2000).

1.2.3 The signalling of CGRP receptor

Schematic illustration of the CGRP receptor mediated intracellular signaling

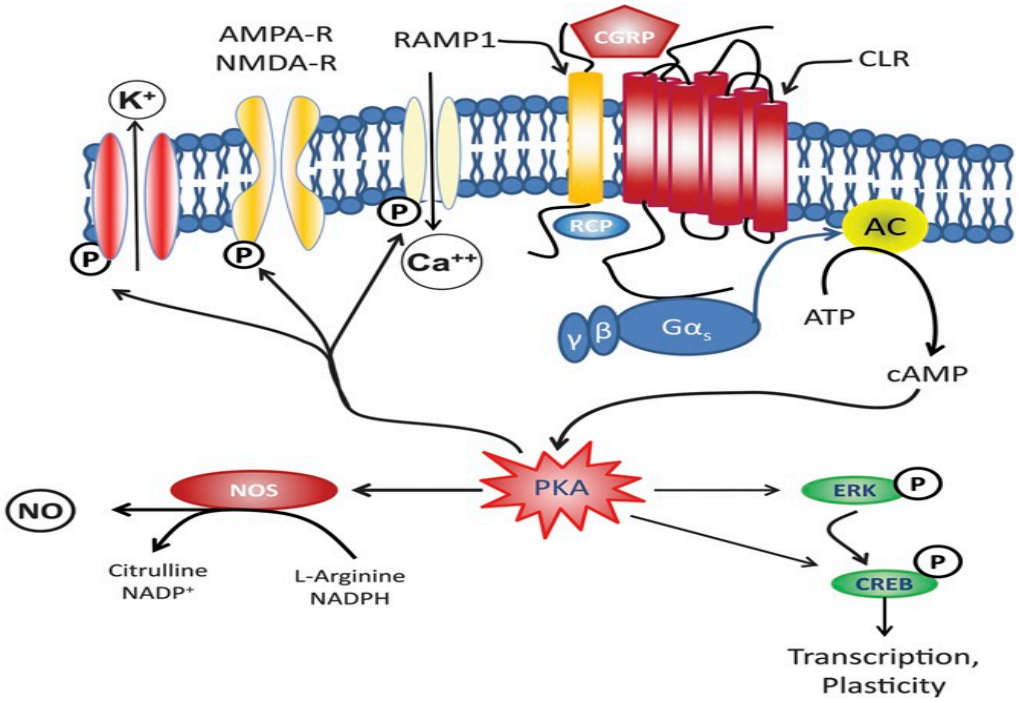


FIGURE 3

The binding of CGRP to the CLR/RAMP1 receptor leads to the activation of various signaling pathways. In fact, the activation of adenylate cyclase (AC) by $G\alpha_S$ leads to increased intracellular cAMP levels, thereby activating protein kinase (PKA), resulting in the phosphorylation of multiple downstream targets, such as the potassium-sensitive ATP channels (K_{ATP} channels), extracellular signal-related kinases (ERKs) and transcription factors such as cAMP-responsive element-binding protein (CREB). The CGRP receptor activation may also generate nitric oxide (NO) via phosphorylation of nitric oxide synthase (NOS). (Iyengar et al., 2017)

1.3 CGRP and CGRP receptor-distribution and function in the trigeminovascular system.

1.3.1 Trigeminovascular system

The trigeminovascular system is part of the system that regulates the cranial vasculature and it is the central element in the transmission of pain involved in headache. This is why it is interesting to watch closely the distribution and the role of CGRP and its receptor in this system *(FIGURE 4 A/B)*.

Schematic illustration of the distribution of CGRP and CGRP receptor in the trigeminovascular system and CNS (central nervous system)	Schematic illustration of the pain pathway in the trigeminovascular system.

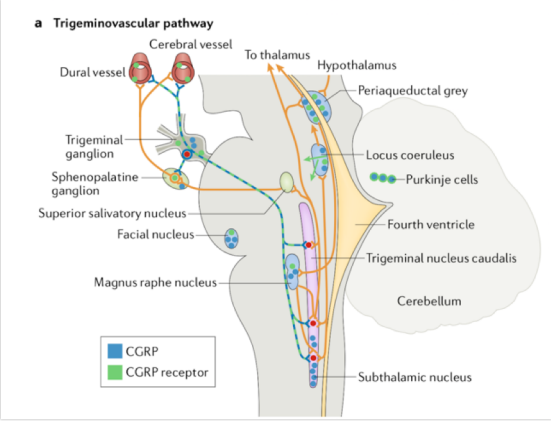


FIGURE 4A

These are the regions, where CGRP (blue) and CGRP receptor (green) have been identified using immunochemistry. Red regions represent cell bodies that express other signaling molecules. (Edvinsson et al., 2018)

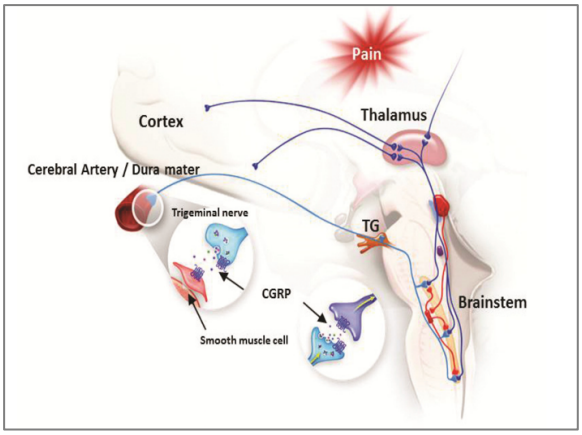


FIGURE 4B

CGRP containing neurons in the TG (trigeminal ganglion) innervate the cerebral blood vessels and the cranial dura mater. Elevated CGRP in the periphery causes vasodilation via action on the smooth muscle of dural vessels. The central terminals of primary afferent fibres of the TG project centrally to the brainstem where CGRP transmits nociceptive inputs to the spinal horns and the trigeminal nucleus caudalis, more precisely to second order neurons. From there the signals are projected to higher cortical regions (Eftekhari, 2013).

1.3.2 Expression in the trigeminal ganglion

The distribution of CGRP and CGRP receptor is almost exclusively found in peripheral sensory nerves. Studies have revealed that α-CGRP is more likely to be found in sensory nerves with cell bodies in the dorsal root ganglion and trigeminal ganglion (Mulderry et al., 1988) (Noguchi et al., 1990).

Bipolar trigeminal sensory neurons have different cell sizes and two types of glial cells, satellite glial cells and Schwann cells. On the whole, C-type sensory neurons release CGRP from the soma or local axon varicosities (Mason et al., 1984) , and CGRP acts on receptors on Aδ-type sensory neurons and satellite glial cells with the aim to regulate pain sensitivty and transmission within the ganglion (*FIGURE 5)*.

Schematic illustration of cells and fibres within the trigeminal ganglion that express either CGRP or the

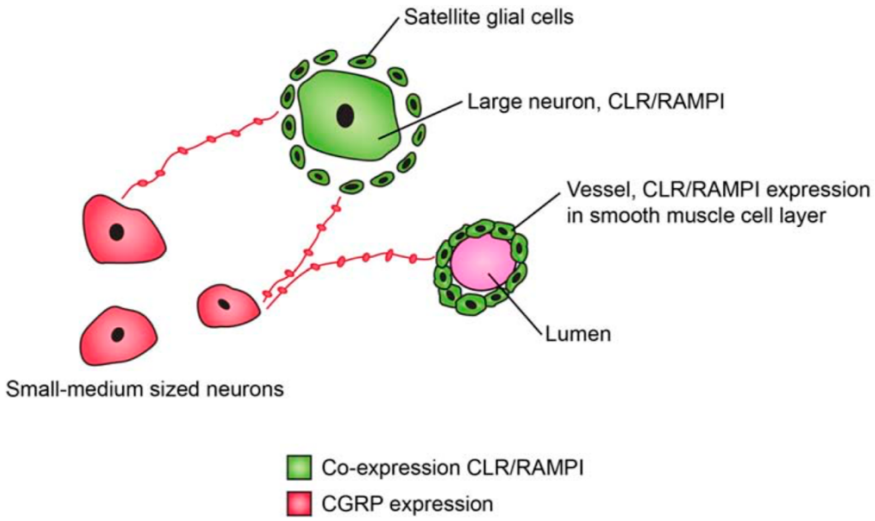


FIGURE 5

CGRP positive neurons have small to medium sized cell bodies and thin, "pearl-like" fibers among the cells also express CGRP (red). The co-expression of receptor components, CLR and RAMP1, takes place in larger cells, satellite glial cells and in the cells of vascular smooth muscle located in blood vessels within the trigeminal ganglion (green). These are sites, where CGRP receptor antagonists may act. (Edvinsson, 2015)

Moreover, CGRP and CGRP receptors have been detected in the peripheral and central branches of trigeminal nerves (Eftekhari et al., 2010). The same expression pattern is observed as in trigeminal ganglion cell bodies (Eftekhari et al., 2013). There is no co-localization of the peptide and the receptor: CGRP can be found in small-diameter, unmyelinated C-fibres that are known to slowly transmit signals of dull, diffuse pain. On the other hand, CGRP receptors have been detected in thicker, myelinated fibres, the so called Aδ-type nociceptive nerves, which signal sharp, acute pain (Eftekhari et al., 2013).

1.3.3 Peripheral targets

- Cerebrovasculature

Trigeminal CGRP nerves almost innervate all vasculatures and CGRP has been shown as one of the most potent vasodilators (Edvinsson et al., 1987). The release of CGRP is thought to act on cerebrovascular smooth muscle cells to increase intracellular cAMP, decrease intracellular Ca^{2+} and cause vasodilatation (Edvinsson et al., 1985, but the peptide has no effect on the cerebrovascular endothelium (Jansen-Olesen et al., 1996). CGRP not only is a potent vasodilator, but also has a pronociceptive action through increased release of substance P and modulation of the synaptic transmission of glutamate (Russel et al., 2014).

- Dura mater

The cranial dura mater is involved in meningeal nociception and stores a wide range of signal molecules that play an important role in migraine (Edvinsson et al., 1981). The neurogenic inflammation in the dura participates in peripheral sensitization and CGRP mediates this process, thanks to its vasodilatory action and its ability to elevate the release of substance P, which in turn induces plasma extravasation (Bernstein et al., 2012). The dura mater also contains numerous mast cells (Orr et al., 1984). Mast cells contain many vasoactive and proinflammatory molecules (such as histamine, serotonin, bradykinin and substance P) (Galli, 1993), that are considered to be involved in migraine pathophysiology (Levy et al., 2007). Studies have revealed that elevated CGRP release may lead to histamine release in rat dura mater via degranulation of mast cells (Ottosson et al., 1997)

1.3.4 Central targets

- Brainstem and spinal cord

As we can see in the *FIGURE 4B* CGRP positive neurons centrally project to the spinal trigeminal nucleus (STN) in the brainstem and in the upper cervical levels of the spinal cord (C_1 and C_2). There CGRP acts on second order neurons, who project the pain signals through ascending pain pathways to higher pain regions (thalamic and cortical regions) via brainstem and midbrain, resulting in perception of pain (Liu et al., 2009). It is believed that migraine is associated with disturbances in the brain and brainstem function (Goadsby et al., 2009). Various clinical studies using positron emission tomography (PET) (Weiller et al., 1995) (Diener, 1997) (Bahra et al., 2001) and functional magnetic resonance imaging (fMRI) (Stankewitz et al., 2011) have shown that brainstem areas in migraine patients are activated

during migraine attacks. As for the spinal cord, stimulations of the C_1 nerve provoked periorbital and frontal pain in migraine patients. These findings suggest that the C_1 nerve might be an important therapeutic target (Johnston et al., 2013).

- CNS

The limited passage of the antibodies and some gepants into the brain speaks against the CNS as a primary target site of action. Therefore the main target is most probably located outside the blood brain barrier (BBB) (Edvinsson, 2015).

1.4 Pathophysiology

1.4.1 Elevated CGRP levels and migraine

Experimental and clinical studies have shown elevated levels of CGRP in plasma and saliva samples taken from patients during migraine attack (Goadsby et al., 1990) (Goadsby et al., 1993) (Bellamy et al., 2006). In addition, infusion of exogenous CGRP can trigger migraine-like headache in patients prone to migraine (Lassen et al., 2002) (Hansen et al., 2010). All these findings underscored the crucial role of CGRP in migraine.

1.4.2 Inhibition of CGRP

- Triptans

Tests have demonstrated that headache relief after sumatriptan coincides with normalization of CGRP levels (Goadsby et al., 1993) (Goadsby et al., 1994). Interestingly, the majority of CGRP positive neurons in the trigeminal ganglion also express $5HT_{1D}$ and $5HT_{1B}$ receptors, indicating that the same cells are targets of triptans (Hou et al., 2001).

- Gepants

Small molecule CGRP antagonists, such as olcegepant (Olesen et al., 2004) and telcagepant (Ho et al., 2008) (Connor et al., 2009) that have been tested in clinical trials had similar efficacy in acute treatment of migraine as triptans. But due to formulation problems (olcegepant) and concerns about the lever toxicity of telcagepant (Ho et al., 2014) the clinical development of gepants has been discontinued. Nevertheless, two chemically different molecules ubrogepant (Voss et al., 2016) and atogepant (Tepper, 2018) and rimegepant (Marcus et al., 2014) are currently undergoing clinical tests and have shown positive results. If these prove to be effective and safe, they will provide an alternative to triptans especially for non-responders and patients with cardiovascular problems.

- mAB

Alternative to small molecules blocking CGRP is the use of monoclonal antibodies (mAB) that bind either CGRP or the receptor.

TABLE 1: Small Molecules versus mAB

	Small Molecules	mAB
Target specifity	low	high
Production	chemically synthesized	in tissue culture
Immunogenicity	no	yes
Size	<1kD	~150kD
Route of administration	oral	parenteral
Cross the BBB	yes/no	no
Pharmacokinetic	more consistent variability	Variable oral absorption
Clearance	liver, kidney	reticulo-endothelial system
Half-life	minutes to hours	1-4 weeks
Binding site	multiple	CGRP receptor or peptide

mAB have various important advantages over gepants in migraine prevention, as they have a highly specific action and they are not metabolized in the liver, they have less potential for liver toxicity. Other relevant differences for preventive treatment are listed in TABLE 1 above (Edvinsson, 2018).

There are three medications available to date: erenumab (Aimovig), fremanezumab (Ajovy), and galcanezumab (Emgality). All are FDA-approved for the prevention of migraine in adults (U.S. Food and Drug Administration, 2018). A fourth, eptinezumab, has completed phase 3 trials, with possible FDA approval in early 2020 (Alder BioPharmaceuticals, Inc., 2019).

TABLE 2: mABs targeting the CGRP pathway

	Erenumab	Fremanezumab	Galcanezumab	Eptinezumab
Type of AB	fully human	humanized	humanized	humanized
Target	CGRP receptor	CGRP	CGRP	CGRP
Type of migraine in trials	EM/CM	EM/CM/cluster headache	EM/CM/cluster headache	EM/CM
Half-life	～3 weeks	40-48 days	28 days	31 days
Dosing and route of action	70mg/140mg monthly S.C	225mg monthly/675mg every 3 months S.C	240mg one month then 120mg monthly S.C	100mg/300mg monthly I.V
Status	FDA approved	FDA approved	FDA approved	late Phase III

EM: Episodic Migraine; CM: Chronic Migraine; S.C: subcutaneous; I.V: Intravenous

All formulations have been tested in numerous phase III trials in CM and EM. All four have shown greater statistical results in reducing migraine days, reducing headache days and in 50% responder rate (the percentage of trial patients achieving a 50% reduction in migraine/headache days) compared with placebo. The reduction of monthly migraine days compared with placebo is on average 2 days for EM and 4-6 days for CM (Edvinsson, 2018).

1.5 Research aims

Based on the introduction above, three research questions have arisen:

-1.5.1 How safe are long term antibody therapies, in particular with CGRP neutralizing antibodies approved for migraine?
-1.5.2 Are preclinical safety data predictive for particular risks of CGRP neutralizing antibodies?
-1.5.3 Is it already possible to estimate the clinical benefit of CGRP neutralizing antibodies and their possible impact on costs and insurance coverage perspective?

2 Methods

Based on literature search, key publications will be reviewed, evaluated for signaling of CGRP, CGRP distribution, the role of CGRP in the pathophysiology of migraine and the pharmacological properties of CGRP inhibition. Together these findings will then focus on the treatment options of currently available monoclonal antibodies. In great detail, the pharmacology, mode of action, pharmacokinetics and clinical outcomes trials will be accomplished for all four mAb. Finally, the evaluation of the long-term safety and efficacy will be the center of this work.

The methods used to answer the above mentioned aims and questions are exclusively based on literature searches and include preclinical and clinical studies. Databases such as PubMed, specialist literature and journals (including online journals) will be used. Supplementary pharmaceutical references are taken from pharmaceutical newspapers or pharmaceutical news (also online). Further information was taken from the official web pages of the products or the web pages of the producing companies. The official web pages of authorities such as the FDA and the EMEA were used for information concerning the approval or the SmPC.
To determinate relevant articles terms such as: erenumab, fremanezumab, galcanezumab, eptinezumab, mAB CGRP, CGRP inhibitors, mAB in migraine. All reviewed articles were in English.

3 Main results

3.1 Risk of long term CGRP block

3.1.1 Safety and tolerability-Erenumab (AMG-334)

TABLE 3: Results from phase II and III trials of erenumab (Aimovig®)

NCT-number protocol and date of completion	**Migraine type**	**Population**	**% with any AE (active vs placebo)**	**% with serious AEs (active vs placebo)**	**Most common AEs**
NCT 02066415 Phase II, randomized double-blind placebo controlled, April 2016 (U.S National Library of Medicine, 2018)	CM	667	140mg: 46,8 vs 39	140mg: 1,1 vs 2,5	Injection site pain, URTI, nausea
NCT 02456740-STRIVE Phase III, randomized double-blind placebo controlled, June 2017 (Goadsby et al., 2017)	EM	955	140mg: 55,5 vs 63	140mg: 3,3 vs 2,19	Injection site pain, URTI, nasopharyngitis, constipation, arthralgia

AE: adverse event; URTI: upper respiratory tract infection

As can be seen from the TABLE 3 above, erenumab proved to be well tolerated. For instance, erenumab- and placebo-treated groups did not differ significantly in the number of AEs. The SmPC suggests in the section entitled 'undesirable effects': in total more than 2 500 patients have been treated with erenumab in registration studies. Of all these, over 1 300 patients were treated for at least 12 months with erenumab. The most common reported adverse drug reactions for 70 mg and 140 mg were injection site reaction (5,6%/4,5%), constipation (1,3%/3,2%), muscle spasms (0,7%/2,0%) and pruritus (1,0%/1,8%). For the most part the reactions were mild or moderate in severity (European Medicines Agency, 2018).

3.1.2 Safety and tolerability-Fremanezumab (TEV-48125)

TABLE 4: Results from phase II and III trials of fremanezumab (Ajovy®)

NCT-number protocol and date of completion	**Migraine type**	**Population**	**% with any AE (active vs placebo)**	**% treatment related AE moderate or severe (active vs placebo)**	**Most common AEs**
NCT 02025556 Phase II, randomized, double-blind, placebo-controlled, March 2015 (Bigal et al., 2015)	EM	297	225mg: 46 vs 56 675mg: 59 vs 56	225mg: 25 vs 27 675mg: 27 vs 27	Injection site pain, erythema
NCT 02629861 Phase III, randomized, double-blind, placebo-controlled, April 2017 (Dodick et al., 2018)	EM	875	225mg: 66 vs 58 675mg: 66 vs 58	225mg: 48 vs 37 675mg: 47 vs 37	Injection site pain, erythema
NCT 02021773 Phase II, randomized, double-blind, double dummy, placebo-controlled,	CM	297	225mg/675mg: 53 vs 40 900mg: 48 vs 40	225mg/675mg 29 vs 17 900mg: 32 vs 17	Injection site pain, pruritus

March 2015 (Bigal et al., 2015-2)					
NCT 02621931 Phase III, randomized, double-blind, placebo-controlled, April 2017 (Silberstein et al., 2017)	CM	1130	225mg: 71 vs 64 675mg: 70 vs 64	225mg: 51 vs 42 675mg: 49 vs 42	Injection site pain, induration, erythema

Just like for erenumab, very few serious adverse events occurred in all the above-mentioned clinical trials (1-2%). As the data from TABLE 4 suggests the most common treatment related AEs were related with the application art (injection). According to the full prescribing information, clinically significant adverse reactions for fremanezumab reported in clinical trials were hypersensitivity reactions, including rash, pruritus, drug hypersensitivity and urticaria. Most of which were mild to moderate, although some led to discontinuation or required a treatment with corticosteroid (U.S Food and Drug Administration, 2018-2).

3.1.3 Safety and tolerability-Galcanezumab (LY2951742)

TABLE 5: Results from phase II and III trials of galcanezumab (Emgality®)

NCT-number protocol and date of completion	**Migraine type**	**Population**	**TEAE (active vs placebo)**	**Most common AEs**
NCT 01625988 Phase II, randomized, double blind, placebo controlled, September 2013 (Dodick et al., 2014)	EM	218	150mg: 72 vs 67	Injection site pain, URTI

NCT 02614183- EVOLVE I Phase III, randomized, double-blind, placebo-controlled, August 2018 (Stauffer et al., 2018)	EM	858	120mg: 65,5 vs 60,4 240mg: 67,7 vs 60,4	Injection site pain, erythema, pruritus, URTI
NCT 02614196- EVOLVE II Phase III, randomized, double-blind, double dummy, placebo-controlled, October 2018 (Skljarevski et al., 2018)	EM	986	120mg: 65 vs 62,3 240mg: 71,5 vs 62,3	Injection site pain, erythema
NCT 02614261- REGAINE Phase III, randomized, double-blind, double dummy, placebo-controlled, Primary March 2017 Estimated 2021 (Detke et al., 2018)	CM	1113	120mg: 58 vs 50 240mg: 57 vs 50	Injection site pain, erythema, pruritus, sinusitis

TEAE: treatment emergent adverse event

The prescribing information suggests that the most common AE reported with galcanezumab in clinical studies were hypersensitivity reactions (rash, urticaria and dyspnea). These reactions can occur days after administration and may be prolonged (U.S Food and Drug Administration, 2018-3).

3.1.4 Safety and tolerability-Eptinezumab (ALD403)

TABLE 6: Results from phase II and III trials of eptinezumab

NCT-number protocol and date of completion	**Migraine type**	**Population**	**TEAE (active vs placebo)**	**Most common AEs**
NCT 01772524 Phase II, randomized, double-blind, placebo-controlled, February 2014 (Dodick et al., 2014)	EM	163	1000mg: 57 vs 52	URTI, UTI, nasopharingitis
NCT 02559895- PROMISE I Phase III, randomized, double-blind, placebo-controlled, December 2017 (Saper et al., 2018-2)	EM	900	30mg: 58 vs 60 100mg: 63 vs 60 300mg: 58 vs 60	URTI, nasopharyngitis, sinusitis
NCT 02974153- PROMISE II Phase III, randomized, double-blind, double dummy, placebo-controlled, April 2018 (Kudrow et al., 2018)	CM	1121	100mg: 38 vs 39 300mg: 44 vs 39	URTI, UTI, nasopharingitis

As we can see in TABLE 6, occurrences of TEAE were same among treatment and placebo groups, suggesting a good tolerability.

All in all, in the phase II and III studies reported here no serious adverse event signals were reported. Taken together, mABs targeting the CGRP pathway seem to offer a safe target-specific preventive

treatment for migraine, while adverse reactions are mainly locally restricted to skin and flu like symptoms. Nevertheless, further investigations are needed in order to determine the long-term safety profile.

3.2 Are preclinical safety data predictive for particular risks of CGRP neutralizing antibodies?

CGRP is broadly expressed throughout the peripheral and enteric nervous system. The peptide being involved in several physiological processes (Russel et al., 2014) and homeostatic responses during pathophysiological conditions (Russel et al., 2014), it is important to consider that an extensive variety of possible adverse events could be caused by blocking CGRP for long duration. As discussed above, mAB against CGRP seem to offer a very promising and unique anti-migraine therapy, nevertheless speculations about long term blocking being dangerous for cardiovascular and cerebrovascular compromised patients exist and are the first and biggest safety concern among others.

3.2.1 Cardiovascular safety concerns

In the cardiovascular system, CGRP can be found in nerve fibres innervating blood vessels (Uddman et al., 1986) and the heart (Opgaard et al., 1995), and participates in the regulation of blood pressure (Russel et al., 2014). Moreover it has also been shown that CGRP has an important role in maintaining cardiovascular homeostasis during ischemic events (Russel et al., 2014). This protective role in pathophysiologic conditions among many others raises concern.

a. CGRP and Hypertension

Hypertension is one of the key factors that can lead to atheroscleoris, cardiac diseases and cerebral insults. One study has demonstrated that CGRP may prevent the onset of hypertension and this protective role is most likely arbitrated via actions at peripheral sites (Smillie et al., 2014). In fact these findings are consistent with the results from a previous study that has shown that the mean arterial pressure and the plasma renin activity were increased in CGRP-knockout mice (Li et al., 2004). This suggests that CGRP reduces blood pressure in pathologic states via modulation of RAAS. Even though CGRP is the most potent peripheral vasodilator acting in the microvasculature it is not involved in physiological regulation of blood pressure (Russel et al., 2014). It is also important to mention the role of CGRP in maintaining cerebral blood flow via autoregulation in chronic hypertension (Wang et al., 2015).

b. CGRP and Cerebral Ischemia

Already back in the eighties, it was shown that CGRP increases blood flow after arterial occlusions (McCulloch et al., 1986). Later, CGRP appeared to have a neuroprotective effect against focal cerebral ischemia by increasing blood flow. And this action takes place via leptin (Zhang et al., 2007) (Zhang et al., 2011). Assays in rats, have demonstrated that the administration of CGRP at the onset of the reperfusion period after experimental cerebral artery occlusion significantly reduced infarct volume. The post ischemic increase of brain edema was also reduced. It is believed that the neuroprotective role of CGRP is either mediated directly by helping to rescue the restoration of blood flow via its vasodilatory properties or indirectly by improving the BBB dysfunction (Liu et al., 2011). CGRP has shown protective actions against ischemia in a range of other organs for example in rat liver (Song et al., 2009).

c. CGRP and the Heart

Various studies in rats and mice suggest that CGRP is released compensatory in response to heart failure (Homma et al., 2014) (Chai et al. 2006) (Li et al. 2002) (Lei et al., 2016). It acts in the heart in a protective manner against ischemia possibly by inducing vasodilation, which in turn decreases the afterload to enhance stroke volume (Bergdahl et al., 1999). CGRP infusion in dogs increased coronary flow and decreased coronary resistance, as well as blood pressure (Joyce et al., 1990). Another study conducted in dogs has shown that CGRP mediates positive inotropy through sympathetic activation. These positive inotropic effects increase stroke volume and ejection fraction (Katori et al., 2005). Comparably in humans, CGRP lowered blood pressure as I mentioned above and protected against heart failure by mediating positive chronotropic and inotropic effects (Gennari et al. 1985) (Saetrum Opgaard et al., 2000). Further studies with evidence for a cardioprotective role, demonstrated: decreased CGRP levels in coronary artery disease (Wang et al., 2012), increased cardiac contractility after i.v. infusion of CGRP in heart failure (Gennari et al. 1990) and implication of CGRP in the response to NO in chronic heart failure (Peng et al., 2012).

To date treatments with anti-CGRP antibodies did not demonstrate any significant cardiovascular related adverse event (see the results above from Phase II and III studies). Furthermore, chronic exposure to anti-CGRP antibodies did not have effects on heart rate or blood pressure in monkeys and rats (Walter et al., 2014) (Zeller et al., 2008). But the above-mentioned protective role raises concern, since migraine patients have been shown to be prone to cardiovascular diseases (Sacco et al. 2014) (Kurth et al., 2016). To date, most of the studies have been conducted on animals or normal, healthy subjects

and do not give further information on how CGRP blockade may affect patients with compromised cardiovascular system. Further long-term studies and post-marketing studies are needed to clarify whether CGRP blocking may exacerbate current cardiovascular diseases or increase the severity of cardiovascular events. These studies should be aimed at clarifying the protective mechanisms and to which extent a blockade of CGRP may cause safety issues. Moreover, including bigger populations and patients with cardiovascular disease history or predestination will allow clinically relevant evaluation of the present concerns. This seems already to be taken in account, as a recent study investigated the effect of erenumab in patients with stable angina (Depre et al., 2018). Results have demonstrated that erenumab did not affect exercise-induced angina or ischemia (Depre et al., 2018). However, we must consider that erenumab currently is the only mAB targeting the receptor and the results may not be same for CGRP peptide blocking. Demystifying whether differences in action and safety exist between mAB against CGRP receptor and CGRP peptide would also be important (MaassenVanDenBrink et al., 2016). Furthermore, the studies must also take in account gender differences because it is suggested that females are more prone to both, strokes and migraine. Hormonal and pathophysiological differences may play an important role in cardiovascular events during CGRP blockade (MaassenVanDenBrink et al., 2016)

3.2.2 Immunological safety concerns

In clinical practice, the use of mAB is associated with a variety of immunological AE. Potential AE could be hypersensitivity, auto immunity and acute infusion complications (Descotes 2009). What is the immunogenic risk for anti CGRP therapies?

CGRP is peptide-ligand-antigen but not an immunogen. Being a ligand, it possesses abilities to form complexes with biomolecules leading to signals. An antigen is a biomolecule that can be recognized by the immune system, while an immunogen is an antigen that can lead to special immune response. CGRP is an antigen, since it can be recognized by mAB but CGRP does not induce an immune response and as such it is not an immunogen (Taylor, 2019). The immunogenic potential of antigens depends on various molecular (stability of shape, size, foreignness…), biologic (responders vs non responders) and administrative (S.C, I.V, dosage…). Based on available data, mAB targeting CGRP are poor immunogens (Taylor, 2019).

Up to date what is known concerning Anti-Drug Antibody (ADA), toxicity, safety and tolerability concerns for ADAs and drug specific immunogenicity?

The data reviewed until now is limited. In phase II and III trials no serious immune mediated adverse reaction (AR) were reported (see results from trials), even though they still contain non-human amino acids (eptinezumab, fremanezumab, galcanezumab are humanized so >90% human amino acids while

erenumab is fully human ~100%), probably because of low risks with humanized antibodies. Still first results (Phase II and Phase III) have shown that a small percentage of patients were recorded with positive ADAs and AEs, in percentages ranging from 1-18% (Taylor, 2019).
It is very difficult to make predictions based on preclinical safety immunological models, current data support low immunogenicity, but this is absolutely limited and a long-term follow-up with sensitive biomarkers could lead to early recognition of specific ADAs. This topic is of big importance because of possible decreased therapy effectiveness and possible immunoallergic hypersensitivity reactions.

3.2.3 Other possible risks and effects.

a. Wound healing

The upcoming risk is more theoretical and has not been mentioned in any clinical trial so far but it could present a possible safety concern, since CGRP has also been associated with enhancement of wound healing (Khalil et al., 1996) (Russel et al., 2014). It is believed that the action relies on the ability of CGRP to promote keratinocytes proliferation (Roggenkamp et al., 2013), enhance revascularization (Mishima et al., 2011) (Majima et al., 2019), reduce expression of tumor necrosis factor α (TNFα) and attenuate macrophage infiltration (Zhang et al., 2010). Considering all these, a result of chronic blocking of CGRP could be alterations in wound healing and high inflammatory responses in skin injuries at the injection site.

b. Gastrointestinal tract (GIT)

The GIT is highly innervated by β-CGRP fibres (Mulderry et al., 1988). And mAB against CGRP are not selective for the α form, but also block β-CGRP. As a matter of fact, mucosal damages were reported in animal studies with mAB targeting CGRP (Peskar et al., 1993) (Reinshagen et al., 1998). CGRP seems to play a role in the maintenance of mucosal integrity of the GIT. Blocking CGRP could therefore trigger inflammatory bowel diseases. Moreover, CGRP is also considered to be involved in motility regulation of GIT, leading to episodes of diarrhea or constipation while blocking CGRP (Bartho et al., 1993). Even though studies have not mentioned monitoring of GIT complications, it may be recommended, because investigations on CGRP KO mice suggested that CGRP agonists may be used for treatment of ulcera (Ohno et al., 2008).

c. Risk of Pre-Eclampsia

CGRP blood levels increase during pregnancy (Yadav et al., 2014) while in women with pre-eclampsia, lower CGRP-levels were recorded (Yadav et al., 2014) (Fei et al., 2012). Even though it is unknown if

these levels are cause or consequence in pre-eclampsia, assuming that the blocking of CGRP might be harmful for the mother and the fetus is very reasonable. Especially in the case of migraine, since most patients diagnosed with migraine are women of child-bearing age (MaassenVanDenBrink et al., 2016).

d. Off-targeting

The precise site of action of blocking CGRP is still unknown. Erenumab being the only CGRP receptor binding antibody, might also bind to other secretin receptors and therefor may exert additional and unforeseeable effects? It was suggested that CGRP may act on amylin receptor. But erenumab CGRP affinity is high and no binding to other receptors was reported, even at highest doses. The affinity was reported to be 5000-fold greater than to AMY_1 and AM receptors (Shi et al. 2016). To date no AEs differing from mAB targeting the ligand have been reported. Eventually, off-targeting may lead to unknown-potential risks in long term observational studies.

To conclude on this part, CGRP is involved in many physiological and pathophysiological actions, therefore it is natural to have concerns about potential risks. Above I listed various safety concerns, some are very important and are also taken into consideration, others are very theoretical. Available data are limited and thus preclude predictions and safety considerations upon long-term usage. The results from clinical trials are very promising and support a well-tolerated new drug family. However, we must await the results from larger and longer trials to evaluate long-term effects.

3.3 Are mAB used for prevention of migraine worth the cost?

3.3.1 Background

Migraine is an extremely debilitating neurological disease and is also very common. It is ranked as the third most prevalent illness worldwide. Obviously, migraine presents a public health issue with serious economic consequences. In fact, in 2015 the costs of treating chronic migraine were estimated more than $5,4 billion in the U.S. Moreover, $36 billion are the total annual estimated costs for health care and lost productivity (Migraine Research Foundation). Various other countries also reported about the socioeconomic impact of migraine. For example, in the UK, according to the Global Burden of Disease study 2016: 23,3% adults are affected by migraine and an average of 11,4 workdays are lost per person per year. Based on this data, the Work Foundation made calculations and concluded that these lost days cost the UK economy about £8,8 billion each year (The Work Foundation).

In France, according to an online survey, migraine patients lose approximately 33 working days per year, which annually costs €3,8 billion to French society (Leiba et al., June 2018). Results have also shown that 58% of patients purchased non-reimbursed medicine for migraine treatment with an average cost of €31,90 per month (Leiba et al., June 2018).

As it was expected, the new CGRP preventive antagonists have high prices. In the USA the price for all three approved agents is approximately $7000 per year (Sparrow et al. 2019). In Austria, the price for Aimovig® is €677/package (～€8000 per year). Due to their cost and mode of administration, prescribing should be almost exclusively for patients with high-frequency episodic or chronic migraine who have failed or who cannot tolerate prior first-line preventive treatments. Industry analysts estimated that about 7 million patients could be suitable for preventive treatment. Leading to a market worth $8 billion up to $10 billion (Court, 2017).

3.3.2 Cost-effectiveness from the Institute for Clinical and Economic Review's (ICER's) point of view.

ICER has released an evidence report in May 2018 assessing the comparative (no preventive treatment or commonly used preventive treatment) clinical effectiveness and the value of 3 CGRP inhibitors for prevention of chronic or episodic migraines (ICER, 2018). Erenumab, fremanezumab and galcanezumab (evidence found to be insufficient) were reviewed for various outcomes such as tolerability, AE, health-related quality of life and such. The report considered existing evidence from clinical trials (in the final reported 18 for EM and 11 for CM). ICER's report estimated that CGRP inhibitors prevented on to three days of migraine per month. When using an estimated price of $5000 (annual net), the report found that the price of the therapies coordinates with the expenses of patient for whom different preventive treatments have failed. All in all, these new CGRG inhibitors were not found to be cost-effective. But it is important to mention that this is valid if used for patients who had not tried other existing, far less expensive preventive treatment. In the case, when the drugs are used for patients for whom previous preventive treatments have failed, ICER estimated an equitable annual price ranging from $3700 to $5300 for erenumab and $37000 to $5200 for fremanezumab. These costs represent a discount of 23% and 46% off the list price, respectively. Moreover, based on an assumed net price of $5000, ICER predicted that of all eligible patients (at least one preventive therapy failed), annually 16% could be treated with erenumab, while 22% could be treated with fremanezumab, without having an impact on the concerns about affordability. The final report was released in July 2018 and it also included results of the California Technology Assessment Forum Panel (June 2018). The meeting discussed the issues regarding the evidences and applying it to practice. Clinical experts came up to the

conclusion that: since mAB targeting CGRP use a new mechanism of action with still unrevealed long-term safety profile, are injectable and patients without benefits are likely to quit treatment, access and affordability are unlikely to become a big concern. ICER on the other side concluded that at this moment it is not issuing an affordability and access alert. But suggested to be careful and to monitor the use of the drugs, as a budget impact is possible (Rodriguez, 2018).

3.3.3 Experts view on the cost effectiveness.

There is a conflict in opinion among the experts, some support the ICER others express disagreement (Hoffman, 2019). According to some experts, the costs might affect already existing treatment inequities because people with low incomes are more likely to suffer from migraine but have less access to this new treatment. They also highlighted ICER's estimation that each migraine-free day with erenumab or fremanezumab treatment costs between $130 and $340 (Burch et al., 2018). Moreover, they also stated that a 75% cost reduction (approximately $2200 per year) is required in order for the whole eligible group of patients to gain access to treatment (Burch et al., 2018).

While other experts believe that results from clinical practice are more relevant than the ones from clinical trials. And they suggested that when determining efficiency, the focus should be on the magnitude of response (Tepper et al., 2019). Furthermore, a paper called 'The ICER myth' has also been released (Winegarden, 2018). This paper underlines that ICER's prices can be used as negotiation tools for health plans, which would have an impact on patients' accessibility to new approved therapies. 'One-size-fits-all Approach', insufficient data, inability to replicate and inadequate metrics are the limitations of the group's reports according to 'The ICER myth'.

To conclude, at this time even making prediction is very difficult, further practical evidences are necessary, especially on heterogenous patient groups in order to demonstrate a net benefit. Determining the cost effectiveness is very complex, various things have to be taken in account such as budget, insurances, health benefit. One thing is sure: this is the first innovation for migraine in many years and many patients have no other options for preventive therapy, they are in need of new treatment possibilities.

4 Discussion

Based on the physiology and pathophysiology of migraine and on the mode of action of new CGRP related drugs, the painful and disabling headache could be a result of dysfunctional signaling in the TG. Currently used treatments for migraine prevention are unspecific and problems such as, little treatment responders, tolerability and AE are very common. With the introduction of mAB targeting the CGRP peptide or the receptor hope of health care professionals and patients rises (Potenza, 2016) (Maasumi et al. 2018). The available data collected so far in phase II and phase III trials are very positive (Edvinsson, 2018). The treatments have shown to be effective with a satisfactory safety profile. Migraine is often associated with various comorbidities and CGRP is implicated in physiological and pathophysiological actions within the whole body. Moreover, little is known about pharmacokinetics and pharmacodynamic of CGRP peptide/receptor antibodies. This far the knowledge about the effects of chronic anti-CGRP treatment is limited. Until now, phase II and III clinical trials did not report any treatment-related serious AE (Edvinsson, 2018). However, it is suggested to keep an eye on cardiovascular reactions. Experts are concerned about the long-term effects regarding this topic (MaassenVanDenBrink et al. 2016). Further investigations are needed in order to wipe out the potential cardiovascular risks. Lastly, as with the majority of antibody therapies, the cost/benefit ratio is also a concern (Hoffmann, 2019) (Sparrow, 2019). Concerning this topic, opinions are divided. For some these novel therapies are not cost-effective and they could have an impact on the budget. For others 'real-life' evidences from clinical practice are required to estimate such things. National and global market studies conducted several studies throughout the years revealing the high socio-economic burden of migraine all over the world (Migraine Research Foundation) (The Work Foundation) (Leiba et al., 2018). High investments in effective migraine-specific treatments (at high price) might not affect the health care budget because, it may reduce the above-mentioned losses. While, investigation of the long-term safety profile will bring up new evidences, for the first time in decades patients will have access to these optimized migraine specific therapies.

So, what are the future implications-which studies should be done?

In many of the above-mentioned studies differences in migraine subtypes were not taken into consideration, age, sex of the patient neither (Giamberardino et al., 2017). It is not known if CGRP antibodies have different effects in various subpopulations (Giamberardino et al., 2017). This is even more relevant when we consider that women in reproductive age are more affected by migraine and also later, they are more likely to have high cardiovascular risks. It would be of great interest to investigate in

future trials how to differentiate responders from non-responders and this way identify the best responder population.

As I already said, migraine is associated with a range of comorbidities and they represent an important aspect to consider. To date no studies have taken in account the common comorbidities such as depression, anxiety, fibromyalgia or the one raising big concern: cardiovascular risks (one study was conducted on angina pectoris patients who suffer from migraine). New effective preventive treatments might also improve the comorbidities but they might also reveal hidden, unexpected and serious reactions, especially on long-term.

It is also crucial to find out the possible differential effects of CGRP antibodies targeting the peptide and targeting the receptor. Until now no different safety profiles have been detected. Maybe patients not benefiting from inhibition of the peptide would benefit from inhibiting the receptor and vis versa. In addition to that, due to the limited knowledge about the exact site of action, off targeting should be taken into consideration. Further studies may give an answer to whether CGRP also acts on other receptors or not and what the consequences are for the CGRP antibody therapy.

Speaking about the long-term effects of blocking CGRP, in particular regarding the cardiovascular concern, they remain unknown and future larger and extended trials should be investigating the effects of these antibodies especially in women with cardiovascular prehistory or high predisposition. One study in patients with angina pectoris did not present any serious effects (Depre et al. 2018). Phase II and phase III trials did not either (Edvinsson, 2018). But the evidences are not sufficient for eliminating possible cardiovascular risks with usage of CGRP antibodies.

In theory the immune system should not be affected by the antibodies targeting CGRP and the receptor because, the humanization reduced this effect (Harding et al., 2010). Yet patients might develop an immune response. Another interesting topic is the development of neutralizing ADA. Even though assays are sensitive, further tests are needed, because with time this effect could have an impact on the efficacy of antibodies in the therapy against migraine.

What about alternative therapies?

The most commonly prescribed preventive therapies are antiepileptic drugs (topiramate), beta blockers (propranolol) and tricyclic antidepressants (amitriptyline), which are labeled for migraine prevention (NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE (NICE), 2012). Many of these drugs have side effects such as fatigue, weight gain, cognitive problems and in particular concerning the majority of migraine patients, a risk of teratogenic effects (Sparrow et al., 2019). The latest innovation in the preventive treatment of migraine was back in 2010, when Botox was approved for CM (Singer, 2010). Botox is among all the only injectable and non-daily therapy. It was shown to have far less side effects in comparison to daily used oral drugs. Results based on cross-trial comparison have shown

that topiramate, Botox and anti-CGRP mABs produce almost similar reduction in monthly days. But patients treated with mABs reported that they had used less acute drugs and some even reported that the pain severity decreased (Sparrow et al. 2019). Concerning the costs, in comparison to beta blockers and other preventive medications for migraine, which exist for a long time now and many generics are available, Botox used to be the most expensive migraine treatment. The price of a vial Botox is about $1100, usually 4 vials are needed per year and injections fees also need to be taken into account. That means that the treatment can cost between $6000 to $10000 per year (Potenza, 2016). Because of high prices most of insurance companies are more likely to encourage patients to try minimum two or more other preventive treatment before receiving Botox injections (Potenza, 2016). This restriction can be very expensive for patients, especially for low-income people, who have first to fail other medications in order to get the opportunity to try Botox. Given the cost, similar restrictions threaten CGRP antibodies. In this case prescribing will almost exclusively be limited for patients who have tried and failed prior treatment. In fact, prescription of anti-CGRP mABs will most probably depend on access, contract agreement and coverage decisions with payers. In the U.S. it is unlikely that all three will be included by the providers (Sparrow et al. 2019). However, considering the population size even a small uptake will lead to considerable profit. An early adoption of anti CGRP mAB is estimated, because of the high demand for new, improved and specific treatment.

It is also interesting to mention the acute space, since oral CGRP-targeted therapies are also in development. The CGRP antagonists ubrogepant and rimegepant are currently in phase III trials for the acute treatment. In late 2018, clinical trials for rimegepant for migraine prophylaxis have been initiated. To date, little is known about its efficacy, but patients often tend to choose oral route over S.C. injections. Anyway, it is efficacy that will be the deciding factor, especially in patients with frequent or severe migraine (Sparrow et al. 2019).

Finally, it is estimated that the migraine market will dramatically grow over the next 10 years, it might even triple in size (from $3,8 billion in 2017 to over $11 billion). And in 2027, drugs targeting CGRP could account for more than $6,5 billion of sales (Sparrow et al. 2019).

As a conclusion, migraine is a big socio-economic burden worldwide and current therapy options for migraine prevention frequently show limitations when it comes to efficacy, inconsistent responses to treatment, high rates of AE and poor tolerability. With the introduction of anti-CGRP mAB we have a possible revolution in migraine therapy. Despite the need for further studies, especially in the long-term safety use, anti-CGRG mAB have shown very promising results. They reduced the number of headache days per month and reduced the use of acute drugs with a positive safety profile. The costs are certainly high. And considering the health care budget these costs will have to be balanced with

the significance of the benefit. Anyway, these monoclonal antibodies seem to offer a new, safe, targeted and effective strategy for migraine prevention, rising hope for many migraineurs. This is why we are awaiting with impatience further results for these new medications.

5 Abstract

ENGLISH

Migraine is a highly disabling neurological disorder that has huge socio-economic impact all over the world. For years the preventive treatment remained very problematic, currently used prophylactic therapy is not specific, adverse events are recurrent and tolerability is low. In 2018 with the approval of three mAB inhibiting CGRP the peptide (fremanezumab, galcanezumab) or its receptor (erenumab), a new era has begun in the treatment of migraine. CGRP plays a crucial role in the pathophysiology of migraine and such therapies are designed specially to act in the trigeminovascular system and block this neuropeptide. These new preventive drugs are more migraine-specific and seem to have a very good safety profile with few or no adverse events. Thus, the following questions have been worked out: i) Are CGRP neutralizing antibodies approved for migraine safe? ii) Are preclinical safety data predictive for particular risks (including long-term safety) of CGRP neutralizing antibodies? iii) Is it already possible to estimate the clinical benefit of CGRP neutralizing antibodies with respect to their possible impact on costs and insurance coverage? Reviewing the current data from clinical trials (phase II and phase III) related to the safety and the tolerability approved CGRP and CGRP receptor targeting mABs are considered as well tolerated and safe. Potential risks may arise from preclinical data highlighting a prominent role of CGRP pathways in microcirculation, post-ischemia improvement and gastrointestinal effects. Finally, I have worked out controversial opinions related to the costs of CGRP and CGRP receptor targeting mABs. However, in the light of these calculations one has to consider the individual improvement in quality of life, the reduction of sickness rate and the improvement of performance at work. All in all, mAB inhibiting the CGRP pathway present a completely new therapeutic approach. Thus, results from large long-lasting trials are eagerly awaited to definitely evaluate the long-term safety and socio-economic aspects of these novel anti-migraine drugs.

DEUTSCH

Migräne ist eine stark behindernde neurologische Erkrankung, die weltweit große sozioökonomische Auswirkungen hat. Die präventive Behandlung blieb über Jahre hinweg sehr problematisch. Die derzeit angewandte prophylaktische Therapie ist nicht spezifisch, unerwünschte Ereignisse treten immer wieder auf und die Verträglichkeit ist gering. Mit der Zulassung von drei monoklonalen Antikörpern, die CGRP hemmen, entweder das Peptid (Fremanezumab, Galcanezumab) oder den Rezeptor (Erenumab), begann im Jahr 2018 eine neue Ära in der Behandlung von Migräne. CGRP spielt eine entscheidende Rolle in der Pathophysiologie von Migräne. Solche Therapien sind speziell darauf ausgelegt, im trigeminovaskulären System zu wirken und dieses Neuropeptid zu blockieren. Diese neuen Präventionsmedikamente sind Migräne-spezifisch und scheinen ein sehr gutes Sicherheitsprofil mit wenigen oder keinen unerwünschten Ereignissen zu haben. Daher wurden die folgenden Fragen ausgearbeitet: i) Sind CGRP-Antikörper, die gegen Migräne zugelassen sind sicher? ii) Sind präklinische Sicherheitsdaten für bestimmte Risiken (einschließlich Langzeitsicherheit) von CGRP-neutralisierenden Antikörpern prädiktiv? iii) Ist es bereits möglich, den klinischen Nutzen von CGRP-neutralisierenden Antikörpern hinsichtlich ihrer möglichen Auswirkungen auf Kosten und Krankenversicherungsschutz abzuschätzen? Die Überprüfung der aktuellen Daten aus klinischen Studien (Phase II und Phase III) in Bezug auf die Sicherheit und Verträglichkeit der genehmigten CGRP- und CGRP-Rezeptor gezielten monoklonalen Antikörper, sie gelten als gut verträglich und sicher. Potentielle Risiken könnten sich aus präklinischen Daten ergeben, dadurch dass, CGRP eine wichtige Rolle bei der Mikrozirkulation und im Gastrointestinalem Trakt ausübt, und der Verbesserung nach einem ischämischen Insult beiträgt. Schließlich habe ich kontroverse Meinungen zu den Kosten von CGRP- und CGRP-Rezeptor gezielten monoklonalen Antikörpern erarbeitet. Diese Berechnungen müssen jedoch die individuelle Verbesserung der Lebensqualität, die Senkung der Krankenquote und die Verbesserung der Arbeitsleistung berücksichtigen. Nichtsdestotrotz bieten monoklonale Antikörper, die den CGRP-Weg hemmem, einen völlig neuen therapeutischen Ansatz. Daher werden die Ergebnisse großer langjähriger Studien mit Spannung erwartet, um die langfristigen Sicherheits- und sozioökonomischen Aspekte dieser neuartigen Anti-Migräne-Medikamente definitiv zu bewerten.

6 List of Abbreviations

5-HT: 5-hydroxytriptamine receptor or serotonin receptor

ADA: Anti-Drug Antibody

AE: adverse event

AMY_1: Amylin receptor 1

AM1 and AM2: adrenomedullin receptor 1 and 2

AMPP: American Migraine Prevalence and Prevention

AMS-I: American Migraine Studies I

AMS-II: American Migraine Studies II

AR: Adverse reaction

BBB : blood brain barrier

CLR: calcitonin receptor-like receptor

CM: Chronic Migraine

CNS: central nervous system

CSD: Cortical spreading depression

EM: Episodic Migraine

FDA : Food and Drug Administration

fMRI : functional magnetic resonance imaging

I.V: intravenous

ICHD: International Classification of Headache Disorders

IHS: International Headache Society

PET: positron emission tomography

RAMP: receptor activity-modifying protein

S.C: subcutaneous

STN: spinal trigeminal nucleus

TEAE: treatment emergent adverse event

TG: trigeminal ganglion

URTI: upper respiratory tract infection

TNFα : Tumor Necrosis Factor α

GIT: Gastro-intestinal tract

7 References

Alder BioPharmaceuticals, Inc. (February 2019). Alder BioPharmaceuticals, Inc. Retrieved March 2019 from https://investor.alderbio.com/news-releases/news-release-details/alder-biopharmaceuticalsr-submits-biologics-license-application

Amara , S.G., Arriza , J.L., Leff, S.E., Swanson, L.W., Evans, R.M., & Rosenfeld , M.G. (1985). Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide. *Science, 229(4718)*, 1094-1097.

Amara , S.G., Jonas, V., Rosenfeld, M.G., Ong, E.S., & Evans, R.M. (1982). Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide product. *Nature, 298(5871)*, 240-244.

Bahra, A., Matharu, M.S., Buchel, C., Frackowiak, R.S., & Goadsby, P.J. (2001). Brainstem activation specific to migraine headache. *Lancet, 357*, 1016-1017.

Bartho, L., Koczan, G., & Maggi, C.A. (1993). Studies on the mechanism of the contractile action of rat calcitonin gene-related peptide and of capsaicin on the guinea-pig ileum: effect of hCGRP (8-37) and CGRP tachyphylaxis. *Neuropeptides, 25*(6), 325-329.

Bartleson, J.D., & Cutrer, F.M. (2010). Migraine update. Diagnosis and treatment. *Minn Med., 93(5)*, 36-41.

Bellamy, J.L., Cady, R.K., & Durham, P.L. (2006). Salivary levels of CGRP and VIP in rhinosinusitis and migraine patients. *Headache., 46(1)*, 24-33.

Bergdahl, A., Valdemarsson, S., Nilsson, T., Sun, X.Y., Hedner, T., & Edvinsson, L. (1999). Dilatory responses to acetylcholine, calcitonin gene-related peptide and substance P in the congestive heart failure rat. *Acta Physiologica Scandinavica, 165*(1), 15-23.

Bernstein, C., & Burstein, R. (2012). Sensitization of the Trigeminovascular Pathway: Perspective and Implications to Migraine Pathophysiology. *J Clin Neurol., 8(2)*, 89-99.

Bigal, M.E., Dodick, D.W., Rapoport, A.M., Silberstein, S.D., Ma, Y., Yang, R., Loupe, P.S., Burstein, R., Newman, L.C., &Lipton, R.B. (2015). Safety, tolerability, and efficacy of TEV-48125 for preventive treatment of high-frequency episodic migraine: a multicentre, randomized, double-blind, placebo-controlled, phase 2b study. *The Lancet Neurology, 14(11)*, 1081-1090.

Bigal, M.E., Edvinsson, L., Rapoport, A.M., Lipton, R.B., Spierings, E.L., Diener, H.C., Burstein, R., Loupes, P.S., Ma, Y., Yang, R., & Silberstein, S.D. (2015-2). Safety, tolerability, and efficacy of TEV-48125 for preventive treatment of chronic migraine: a multicentre, randomised, double-blind, placebo-controlled, phase 2b study. *The Lancet Neurology, 14(11)*, 1091-1100.

Boehnke, C., Reuter, U., Flach, U., Schuh-Hofer, S., Einhäupl, K.M., & Arnold, G. (2004). High-dose riboflavin treatment is efficacious in migraine prophylaxis: an open study in a tertiary care centre. *Eur J Neurol., 11(7)*, 475-477.

Born , W., & Fischer, J.A. (2010). The calcitonin peptide family: what can we learn from receptor knock out and transgenic mice. In: Hay L.D., Dickerson M.I. editors. The calcitonin gene-related peptide family: form, function and future perspectives. *Dordrecht: Springer*, 75-86.

Bose, P., & Goadsby, P.J. (2016). The migraine postdrome. *Current Opinion in Neurology, 29(3)*, 299-301.

Burch, R., & Rayhill, M. (2018). New preventive treatments for migraine. *BMJ (Clinical Research Ed.), 361*, k2507.

Burch, R., Rizzoli, P., & Loder, E. (2018). The Prevalence and Impact of Migraine and Severe Headache in the United States: Figures and Trends From Government Health Studies. *Headache, 58(4)*, 496-505.

Buzzi, M.G., Cologno, D., Formisano, R., & Rossi, P. (2005). Prodromes and the early phase of the migraine attack: therapeutic relevance. *Functional Neurology, 20 (4)*, 179-183.

Chai, N.C., Peterlin, B.L., & Calhoun, A.H. (2014). Migraine and estrogen. *Current Opinion in Neurology, 27(3)*, 315-358.

Chai, W., Mehrotra, S., Jan Danser, A.H., & Schoemaker, R.G. (2006). The role of calcitonin gene-related peptide (CGRP) in ischemic preconditioning in isolated rat hearts. *European Journal of Pharmacology, 531*(1-3), 246-253.

Charles, A. (2009). Advances in the basic and clinical science of migraine. *Ann Neurol, 65(5)*, 491-498.

Charles, A., & Brennan, K.C. (2010). The neurobiology of migraine. *Handb Clin Neurol , 97*, 99-108.

Colman , I., Friedman, B.W., Brown, M.D., Innes, G.D., Grafstein, E., Roberts, T.E., & Rowe, B.H. (2008). Parenteral dexamethason for acute severe migraine headache: meta-analysis of randomized controlled trials for preventing recurrence. *BMJ, 336(7657)*, 1359-1361.

Connor, K.M., Shapiro, R.E., Diener, H.C., Lucas, S., Kost, J., Fan, X., Fei, K., Assaid, C., Lines, C. & Ho, T.W. (2009). Randomized, controlled trial of telcagepant for the acute treatment of migraine. *Neurology, 73(12)*, 970-977.

Cutrer, F.M. (2010). Pathophysiology of migraine. *Semin Neurol, 30(2)*, 120-130.

Depre, C., Antalik, L., Starling, A., Koren, M., Eisele, O., Lenz, R.A., & Mikol, D.D. (2018). A Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Effect of Erenumab on Exercise Time During a Treadmill Test in Patients With Stable Angina. *Headache, 58*(5), 715-723.

Descotes, J. (2009). Immunotoxicity of monoclonal antibodies. *mAbs, 1*(2), 104-111.

Derry, S., & Moore, A.R. (2013b). Paracetamol (acetaminophen) with or without an antiemetic for acute migraine headaches in adults. *The Cochrane Database of Systematic Reviews, 4*, CD008040.

Derry, S., Rabbie, R., & Moore, A.R. (2013). Diclofenac with or without an antiemetic for acute migraine headaches in adults. *The Cochrane Database of Systematic Reviews, 4*, CD008783.

Detke, H.C., Goadsby, P.J., Wang, S., Friedman, D.I., Selzler, K.J., & Aurora, S.K. (2018). Galcanezumab in chronic migraine: The randomized, double-blind, placebo-controlled REGAIN study. *Neurology, 91(24)*, 2211-2221.

Diener, H. C. (1997). Positron emission tomography studies in headache. *Headache, 37*, 622-625.

Dodick, D.W., Goadsby, P.J., Silberstein, S.D., Lipton, R.B., Olesen, J., Ashina, M., Wilks, K., Kudrow, D., Kroll, R., Kohrman, B., Bargar, R., Hirman, J. & Smith, J. (2014). Safety and efficacy of ALD403, an antibody to calcito-nin gene-related peptide, for the prevention of frequent episodic migraine: a randomised, double-blind, placebo-controlled, exploratory phase 2 trial. *Lancet Neurol., 13(11)*, 1100-1107.

Dodick, D.W., Goadsby, P.J., Spierings, E.L., Scherer, J.C., Sweeney, S.P., & Grayzel, D.S. (2014). Safety and efficacy of LY2951742, a monoclonal an-tibody to calcitonin gene-related peptide, for the preven-tion of migraine: a phase 2, randomised, double-blind, placebo-controlled study. *Lancet Neurol., 13(9)*, 885-892.

Dodick, D.W., Stephen, D., Silberstein, M.D., Bigal, M.E., Yeung, P.P., Goadsby, P.J., Blankenbiller, T., Grozinski-Wolff, M., Yang, R., Ma, Y., & Aycardi, E. (2018). Effect of Fremanezumab Compared with Placebo of Prevention of Episodic Migraine A Randomized Clinical Trial. JAMA, 319(19), 1999-2008.

Dodick, D.W., Turkel, C.C., DeGryse, R.E., Aurora, S.K., Silberstein, S.D., Lipton, R.B., Diener, H.C, & Brin, M. F. (2010). OnabotulinumtoxinA for treatment of chronic migraine: pooled results from double-blind, randomized, placebo-controlled phases of the PREEMPT clinical program. *Headache, 50(6)*, 921-936.

Edvinsson, L. (2015). CGRP receptor antagonists and antibodies against CGRP and its receptor in migraine treatment. *Br J Clin Pharmacol., 80(2)*, 193-199.

Edvinsson, L. (2018). The CGRP Pathway in Migraine as a Viable Target for Therapies. *Headache, 58*, 33-47.

Edvinsson, L., & Uddman, R. (1981). Adrenergic, cholinergic and peptidergic nerve fibres in dural mater involvement in headache? *Cephalalgia, 1(4)*, 175-179.

Edvinsson, L., Ekman, R., Jansen, I., McCulloch, J., & Uddman, R. (1987). Calcitonin gene-related peptide and cerebral blood vessels: distribution and vasomotor effects. *J Cereb Blood Flow Metab., 7(6)*, 720-728.

Edvinsson, L., Fredholm, B.B., Hamel, E., Jansen, I., & Verrecchia, C. (1985). Perivascular peptides relax cerebral arteries concomitant with stimulation of cyclic adenosine monophosphate accumulation or release of an endothelium-derived relaxing factor in the cat. *Neurosci. Lett., 58*, 213-217.

Edvinsson, L., Haanes, K.A., Warfvinge, K., & Krause, D.N. (2018). CGRP as the target of new migraine therapies-successful translation from bench to clinic. *Nat Rev Neur, 14*, 338-350.

Eftekhari , S., Salvatore, C.A., Calamari, A., Kane, S. A., Tajti, J., & Edvinsson, L. (2010). Differential distribution of calcitonin gene-related peptide and its receptor components in the human trigeminal ganglion. *Neuroscience, 169(2)*, 683-696.

Eftekhari , S., Warfvinge, K., Blixt, F.W., & Edvinsson, L. (2013). Differentiation of nerve fibers storing CGRP and CGRP receptors in the peripheral trigeminovascular system. *J. Pain, 14*, 1289-1303.

Eftekhari, S. (2013). *Huvudvarkssallskapet-Swedish Headache Society.* Retrieved March 2019, from Doctoral dissertation: Distribution of CGRP and CGRP receptor in the trigeminovascular system and CNS.: http://www.huvudvarkssallskapet.se/upload/fil_4.pdf

Emma Court. (2017, Jun 9). New migraine drugs have promise — and a $8,500 price tag; CGRP drugs work to prevent migraines before they occur, and could be on the market as early as next year. *MarketWatch* Retrieved from https://global.factiva.com/en/du/article.asp?accessionno=MRKWC00020170609ed6900335

European Medicines Agency. (2018, August). *ema.europa.eu*. Retrieved March 2019, from https://www.ema.europa.eu/en/documents/product-information/aimovig-epar-product-information_en.pdf

Evans, B.N., Rosenblatt, M.I., Mnayer, L.O., Oliver, K.R., & Dickerson, I.M. (2000). CGRP-RCP, a novel protein required for signal transduction at calcitonin gene-related peptide and adrenomedullin receptors. *J Biol Chem, 275(40)*, 31438-31443.

Fei, X., Hongxiang, Z., Qi, C., & Daozhen, C. (2012). Maternal plasma levels of endothelial dysfunction mediators including AM, CGRP, sICAM-1 and tHcy in pre-eclampsia. *Advances in Clinical and Experimental Medicine : Official Organ Wroclaw Medical University, 21*(5), 573-579.

Ferrari, M.D., Roon, K.I., Lipton , R.B., & Goadsby, P.J. (2001). Oral triptans (serotonin 5-HT(1B/1D)agonists) ina cute migraine treatment: a meta-analysis of 53 trials. *Lancet, 358(9294)*, 1668-1675.

Fux, C., & Matzik, S. (June 2018). Rerieved from: https://www.netdoktor.de/krankheiten/migraene/

Galli, S. J. (1993). New concepts about the mast cell. *N Engl J Med, 328*, 257-265.

Gennari, C., & Fischer, J.A. (1985). Cardiovascular action of calcitonin gene-related peptide in humans. *Calcified Tissue International, 37*(6), 581-584.

Gennari, C., Nami, R., Agnusdei, D., & Fischer, J. A. (1990). Improved cardiac performance with human calcitonin gene related peptide in patients with congestive heart failure. *Cardiovascular Research, 24*(3), 239-241.

Giamberardino, M.A., Affaitati, G., Costantini, R., Cipollone, F., & Martelletti, P. (2017). Calcitonin gene-related peptide receptor as a novel target for the management of people with episodic migraine: current evidence and safety profile of erenumab.*Journal of Pain Research, 10*, 2751-2760.

Gilmore, B., & Michael, M. (2011). Treatment of acute migraine headache. *American Family Physician, 83(3)*, 271-280.

Goadsby , P.J., Charbit, A.R., Andreou, A.P., Akerman, S., & Holland, P.R. (2009). Neurobiology of migraine. *Neuroscience, 161*, 327-341.

Goadsby , P.J., Edvinsson, L., & Ekman, R. (1990). Vasoactive peptide release in the extracerebral circu-lation of humans during migraine headache. *Ann Neurol, 28(2)*, 183-187.

Goadsby, P.J. (2009). Pathophysiology of migraine. *Neurol Clin, 27(2)*, 335-360.

Goadsby, P.J., & Edvinsson, L. (1993). The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. *Ann Neurol, 33(1)*, 48-56.

Goadsby, P.J., & Edvinsson, L. (1994). Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies. *Brain, 117*, 427-434.

Goadsby, P.J., Reuter, U., Hallström, Y., Broessner, G., Bonner, J.H., Zhang, F., Sapra, S., Picard, H., Mikol, D.D., & Lenz, R.A. (2017). A controlled Trial of Erenumab for Episodic Migraine. N Eng J Med, 377, 2123-2132.

Gursoy-Ozdemir, Y., Qiu, J., Matsuoka, N., Bolay, H., Bermpohl, D., Jin, H., Wang, X., Rosenberg, G.A., Lo, E.H., & Moskowitz, M.A. (2004). Cortical spreading depression activates and upregulates MMP-9. *J Clin Invest, 113(10)*, 1447-1455.

Hagen, K., Zwart, J.A., Vatten, L., Stovner, L.J., & Bovim, G. (2000). Prevalence of migraine and non-migrainous heada-che-head-HUNT, a large population-based study. *Cephalalgia, 20(10)*, 900-906.

Hansen , J.M., Hauge, A.W., Olesen, J., & Ashina, M. (2010). Calcitonin gene-related peptide triggers migraine-like attacks in patients with migraine with aura. *Cephalalgia, 30(10)*, 1179-1186.

Harding, F.A., Stickler, M.M., Razo, J., & DuBridge, R.B. (2010). The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *mAbs, 2*(3), 256-265.

Headache Classification Subcommitee of the IHS. (2018). The international classification of headache disorders: 3rd edition. *38(1)*, 18-28.

Health Union. (2018, August). *Migraine*. Retrieved from https://migraine.com/infographic/migraine-then-now/

Henry, P., Auray, J.P., Gaudin, A.F., Dartigues, J.F., Duru, G., Lantéri-Minet, M., Lucas, C., Pradalier, A., Chazot, G., & El Hasnaoui, A. (2002). Prevalence and clinical characteristics of migraine in France. *Neurology, 59(2)*, 232-237.

Henry, P., Michel, P., Brochet, B., Dartigues, J.F., Tison, S., & Salamon, R. (1992). A nationwide survey of migraine in France: prevalence and clinical features in adults. GRIM. *Cephalalgia, 12(4)*, 229-237.

Hering, R., & Kuritzky, A. (1992). Sodium valproate in the prophylactic treatment of migraine: a double-blind study versus placebo. *Cephalalgia, 12(2)*, 81-84.

Ho, T.W., Connor, K.M., Zhang, Y., Pearlman, E., Koppenhaver, J., Fan, X., Lines, C., Edvinsson, L., Goadsby, P.J., & Michelson, D. (2014). Randomized controlled trial of the CGRP receptor antagonist telcagepant for migraine prevention. *Neurology, 83*, 958-966.

Ho, T.W., Ferrari, M.D., Dodick, D.W., Galet, V., Kost, J., Fan, X., Leibensperger, H., Froman, S., Assaid, C., Lines, C., Koppen, H. et al. (2008). 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, 372*, 2115-2123.

Hoffman Matt. (2019, January 19). For patients with migraine are CGRP inhibitors worth the cost? Retrieved from https://www.neurologylive.com/clinical-focus/patients-migraine-cgrp-inhibitors-worth-cost

Homma, S., Kimura, T., Sakai, S., Yanagi, K., Miyauchi, Y., Aonuma, K., & Miyauchi, T. (2014). Calcitonin gene-related peptide protects the myocardium from ischemia induced by endothelin-1: intravital microscopic observation and (31)P-MR spectroscopic studies. *Life Sciences, 118*(2), 248-254.

Hou, M., Kanje, M., Longmore, J., Tajti, J., Uddman, R., & Edvinsson, L. (2001). 5-HT(1B) and 5-HT(1D) receptors in the human trige-minal ganglion: co-localization with calcitonin gene-related peptide, substance P and nitric oxide synthase. *Brain Res, 909*, 112-120.

ICER. (2018). Institute for Clinical and Economic Review Finds Price of Erenumab Aligns with Clinical Benefit in Patients for Whom Other Therapies Have Failed to Prevent Migraines. Retrieved from https://icer-review.org/announcements/cgrp-evidence-report/

Iyengar, S., Ossipov, M.H., & Johnsona, K.W. (2017). The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine. *Pain J, 158*, 543-549.

Jansen-Olesen, I., Mortensen, A., & Edvinsson, L. (1996). Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilatation of human cerebral arteries concomitant with activation of adenylyl cyclase. *Cephalalgia, 16*, 310-316.

Jensen, R., Brinck, T., & Olesen, J. (1994). Sodium valproate has a prophylactic effect in migraine without aura: a triple blind, placebo-controlled crossover study. *Neurology, 44(4)*, 647-651.

Johnston, M.M., Jordan , S.E., & Charles, A.C. (2013). Pain referral patterns of the C1 to C3 nerves: Implications for headache disorders. *Ann Neurol., 74*, 145–148.

Joyce, C.D., Prinz, R.A., Thomas, J.X., Fiscus, R.R., Wang, X.A., Djuricin, G., & Jacobs, H.K. (1990). Calcitonin gene-related peptide increases coronary flow and decreases coronary resistance. *The Journal of Surgical Research, 49*(5), 435-440.

Karsan, N., Palethorpe, D., Rattanawong, W., Marin, J. C., Bhola, R., & Goadsby, P.J. (2018). Flunarizine in migraine-related headache prevention: results from 200 patients treated in the UK. *Eur J Neurol., 25(6)*, 811-817.

Katori, T., Hoover, D.B., Ardell, J.L., Helm, R.H., Belardi, D.F., Tocchetti, C.G., Forfia, P.R., Kass, D.A., & Paolocci, N. (2005). Calcitonin gene-related peptide in vivo positive inotropy is attributable to regional sympatho-stimulation and is blunted in congestive heart failure. *Circulation Research, 96*(2), 234-243.

Kelman, L. (2005). Migraine pain location: a tertiary care study of 1283 migraineurs. *Headache , 45(8)*, 1038-1047.

Kelman, L. (2006). The postdrome of the acute migraine attack. *26(2)*, 214-220.

Kelman, L. (2007). The triggers or precipitants of the acute migraine attack. *Cephalalgia, 27(5)*, 394-402.

Khalil, Z., & Helme, R. (1996). Sensory peptides as neuromodulators of wound healing in aged rats. *The Journals of Gerontology.Series A, Biological Sciences and Medical Sciences, 51*(5), 354.

Kudrow, D., Diamond, M., McGill, L., Cady, R., Bhattacharya, S., Biondi, D., & Smith, J. (2018, April). *alderbio.com.* Retrieved March 2019, from https://investor.alderbio.com/events-and-presentations: https://investor.alderbio.com/static-files/18217812-fc82-4f68-8da4-b94091d3a3eb

Kurth, T., Winter, A. C., Eliassen, A. H., Dushkes, R., Mukamal, K. J., Rimm, E. B., Willett, W.C., Manson, J.E. & Rexrode, K.M. (2016). Migraine and risk of cardiovascular disease in women: prospective cohort study. *BMJ (Clinical Research Ed.), 353*, i2610.

LaGreve's , P., Andersson, K., & Silberring, J. (1997). Isolation and identification of CGRP C-terminal fragments in the rat spinal cord. *Neuropeptides, 31*, 19-23.

Lampl , C., Katsarava, Z., Diener, H.C., & Limmroth, V. (2008). Lamotrigine reduces migraine aura and migraine attacks in patients with migraine with aura. *J. Neurol. Neurosurg. Psychiatry , 76(12)*, 1730-1732.

Lassen, L.H., Haderslev, P.A., Jacobsen, V.B., Iversen, H.K., Sperling, B., & Olesen, J. (2002). CGRP may play a causative role in migraine. *Cephalalgia, 22(1)*, 54-61.

Launer , L.J., Terwindt, G.M., & Ferrari, M.D. (1999). The prevalence and characteristics of migraine in a population-based cohort: the GEM study. *Neurology, (53(3)*, 537-542.

Levy , D., Burstein, R., Kainz, V., Jakubowski, M., & Strassman, A. M. (2007). Mast cell degranulation activates a pain pathway underlying migraine headache. *Pain , 130*, 166-176.

Lei, J., Zhu, F., Zhang, Y., Duan, L., Lei, H., & Huang, W. (2016). Transient Receptor Potential Vanilloid Subtype 1 Inhibits Inflammation and Apoptosis via the Release of Calcitonin Gene-Related Peptide in the Heart after Myocardial Infarction. *Cardiology, 134*(4), 436-443.

Leiba, G., Bastien, A., Cayre, F., Mahieu, N., & Aly, S. (June 2018). Socio-economic impact of severe migraine in France: study in patients with at least 8 days of headaches per month. Paper presented at the *4th Congress of the European Academy of Neurology, Lisbon, Portugal, 25*(S2) EP01050.

Li, J., Zhao, H., Supowit, S.C., DiPette, D.J., & Wang, D. (2004). Activation of the renin-angiotensin system in alpha-calcitonin gene-related peptide/calcitonin gene knockout mice. *22(7)*, 1345-1349.

Li, Y.J., & Peng, J. (2002). The cardioprotection of calcitonin gene-related peptide-mediated preconditioning. *European Journal of Pharmacology, 442*(3), 173-177.

Lipton , R.B., Stewart, W.F., & Simon, D. (1998). Medical consultation for migraine: results from the American Migraine Study. *Headache, 38*, 87-96.

Lipton, R.B., Bigal, M.E., Freitag, F., Reed, M.L., & Stewart, W.F. (2007). Migraine prevalence, disease burden, and the need for preventive therapy. *Neurology, 68(5)*, 343-349.

Lipton, R.B., Diamond, S., Reed, M., Diamond, M.L., & Stewart, W.F. (2001b). Migraine diagnosis and treatment: results from the American Migraine Study II. *Headache, 41*, 638-645.

Lipton, R.B., Stewart, W.F., Diamond, S., Diamond, M.L., & Reed, M. (2001a). Prevalence and burden of migraine in the United States: data from the American Migraine Study II. *Headache, 41(7)*, 646-657.

Liu, Z., Liu, Q., Cai, H., Xu, C., Liu, G., & Li, Z. (2011). Calcitonin gene-related peptide prevents blood-brain barrier injury and brain edema induced by focal cerebral ischemia reperfusion. *Regulatory Peptides, 171*(1-3), 19-25.

Liu, Y., Broman, J., Zhang, M., & Edvinsson, L. (2009). Brainstem and thalamic projections from a craniovascular sensory nervous centre in the rostral cervical spinal dorsal horn of rats. *Cephalalgia , 29*, 935-948.

Lynn, J.D., Newton, H.B., & Rae-Grant, A. (2004). *The 5-minute Neurology Consult.* Lippincott Williams and Wilkins.

MaassenVanDenBrink, A., Meijer, J., Villalon, C.M., & Ferrari, M.D. (2016). Wiping Out CGRP: Potential Cardiovascular Risks. *Trends in Pharmacological Sciences, 37*(9), 779-788.

Maasumi, K., Michael, R. L., & Rapoport, A. M. (2018). CGRP and Migraine: The Role of Blocking Calcitonin Gene-Related Peptide Ligand and Receptor in the Management of Migraine. *Drugs*, 78(9), 913-928.

Majima, M., Ito, Y., Hosono, K., & Amano, H. (2019). *CGRP/CGRP Receptor Antibodies: Potential Adverse Effects Due to Blockade of Neovascularization?*

Malick, A., & Burstein , R. (2000). Peripheral and central sensitization duing migraine. *Funct Neurol, 15(3)*, 28-35.

Marcus, R., Goadsby, P.J., Dodick, D., Stock, D., Manos, G., & Fischer, T.Z. (2014). BMS-927711 for the acute treatment of migraine: a double-blind, randomized, placebo controlled, dose-ranging trial. *Cephalalgia, 34(2)*, 114-125.

Mason , R.T., Peterfreund, R.A., Sawchenko, P.E., Corrigan, A.Z., Rivier, J.E., & Vale, W.W. (1984). Release of the predicted calcitonin gene-related peptide from cultured rat trigeminal ganglion cells. *Nature, 308(5960)*, 653-655.

Mathew, N. T., Rapoport, A., Saper, J., Magnus, L., Klapper, J., Ramadan, N., Stacey, B. & Tepper, S. (2001). Efficacy of gabapentin in migraine prophylaxis. *Headache, 41(2)*, 119-128.
McCulloch, J., Uddman, R., Kingman, T. A., & Edvinsson, L. (1986). Calcitonin gene-related peptide: functional role in cerebrovascular regulation. *Proceedings of the National Academy of Sciences of the United States of America, 83*(15), 5731-5735

McLatchie, L.M., Fraser, N.J., Main, M.J., Wise, A., Brown, J., Thompson, N., Solari, R., Lee, M.G. & Foord, S.M. (1998). RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. *Nature, 393 (6683)*, 333-339.

Migraine Research Foundation. Migraine is an extraordinarily prevalent neurological disease, affecting 39 million men, women and children in the U.S. and 1 billion worldwide. Retrieved from https://migraineresearchfoundation.org/about-migraine/migraine-facts/

Mishima, T., Ito, Y., Hosono, K., Tamura, Y., Uchida, Y., Hirata, M., Suzsuki, T., Amano, H., Kato, S., Kurihara, Y., Kurihara, H., et al. (2011). Calcitonin gene-related peptide facilitates revascularization during hindlimb ischemia in mice. *American Journal of Physiology. Heart and Circulatory Physiology, 300*(2), 431.

Mulderry, P.K., Ghatei, M.A., Spokes, R.A., Jonhs, P.M., Pierson , A.M., Hamid, Q.A., Kanse, S., Amara, S.G., Burrin, J.M., Legon, S., et al. (1988). Differential expression of a-CGRP and b-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. *Neuroscience, 25*, 195-205.

NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE (NICE) (2012). Headaches in over 12s:diagnosis and management. Retrieved from https://www.nice.org.uk/guidance/cg150

Noguchi, K., Senba, E., Morita, Y., Sato, M., & Tohyama, M. (1990). Co-expression of alpha-CGRP and beta-CGRP mRNAs in the rat dorsal root ganglion cells. *Neurosci Lett, 108*, 1-5.

Ohno, T., Hattori, Y., Komine, R., Ae, T., Mizuguchi, S., Arai, K., Saeki, T., Suzuki, T., Hosono, K., Hayashi, I., Oh-Hashi, Y., et al. (2008). Roles of calcitonin gene-related peptide in maintenance of gastric mucosal integrity and in enhancement of ulcer healing and angiogenesis. *Gastroenterology, 134*(1), 215-225. Ohno T, Hattori Y, Komine R, Ae T, Mizuguchi S, Arai K, Majima M.

Olesen, J., Diener, H.C., Husstedt, I.W., Goadsby, P. J., Hall, D., Meier, U., Pollentier, S. & Lesko, L.M. (2004). Calcitonin Gene–Related Peptide Receptor Antagonist BIBN 4096 BS for the Acute Treatment of Migraine. *N Eng J Med, 350*, 1104-1110.

Opgaard, O. S., Gulbenkian, S., Bergdahl, A., Barroso, C. P., Andrade, N. C., Polak, J. M., Queiroz e Melo, J.Q; & Edvinsson, L. (1995). Innervation of human epicardial coronary veins: im-munohistochemistry and vasomotility. *29(4)*, 463-468.

Orr , E. L., & Pace, K. R. (1984). The significance of mast cells as a source of histamine in the mouse brain. *J Neurochem, 42*, 727-732.

Ottosson , A., & Edvinsson, L. (1997). Release of histamine from dural mast cells by substance P and calcitonin gene-related peptide. *Cephalalgia, 17*, 166-174.

Padilla, B.E., Cottrell, G.S., Roosterman, D., Pikios, S., Muller, L., Steinhoff, M., & Bunnett, N.W. (2007). Endothelin converting enzyme-1 regulates endosomal sorting of calcitonin receptor-like receptor and beta-arrestins. *J Cell Biol, 179*, 981-997.

Pavlovic, J.M., Buse, D.C., Sollars, C.M., Haut, S., & Lipton , R.B. (2014). Trigger factors and premonitory feature of migraine attacks:summary of studies. *Headache, 54(10)*, 1670-1679.

Peng, L.M., Chen, X.P., Sun, J., Guo, Y. J., Li, L., Mo, L., Xie, W., Li, Y.J., Yang, T.L. & Li, C.C. (2012). Influence of ALDH2 Glu504Lys polymorphism on nitroglycerin response in chronic heart failure and involvement of Calcitonin Gene Related Peptide (CGRP). *International Journal of Clinical Pharmacology and Therapeutics, 50*(10), 701-711.

Peskar, B. M., Wong, H. C., Walsh, J. H., & Holzer, P. (1993). A monoclonal antibody to calcitonin gene-related peptide abolishes capsaicin-induced gastroprotection. *European Journal of Pharmacology, 250*(1), 201-203.

Potenza Alessandra. (2016, May 24,). New migraine drugs promise relief — but at a steep price. Retrieved from https://www.theverge.com/2016/5/24/11712554/migraine-drug-treatment-headache-cost-pharmaceutical-health-insurance

Poyner , D. R., Sexton, P. M., Marshall, I., Smith, D. M., Quirion , R., Born, W., Muff, R., Fischer, J.A. & Foord, S. M. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin and calcitonin receptors. *Pharmacol Rev, 2002*(54), 233-246.

Rabbie, R., Derry, S., Moore, A.R., & McQuay, H.J. (2010). Ibuprofen with or without an antiemetic for acute migraine headaches in adults. *4*, CD008039.

Rabkin, R., Stables, D.P., Levin, N.V., & Suzman, M.M. (1966). The prophylactic value of propranolol in angina pectoris. *Am J Cardiol., 18(3)*(370-383).

Radat, F. (2013). Stress and migraine. *Revue Neurologique, 169(5)*, 406-412.

Rasmussen , B.K., & Olesen, J. (1992). Migraine with aura and migraine without aura: an epidemiological study. *Cephalalgia, 12(4)*, 221-228.

Reinshagen, M., Flamig, G., Ernst, S., Geerling, I., Wong, H., Walsh, J. H., Eysselein, V.E. & Adler, G. (1998). Calcitonin gene-related peptide mediates the protective effect of sensory nerves in a model of colonic injury. *The Journal of Pharmacology and Experimental Therapeutics, 286*(2), 657-661.

Rodriguez Alison. (2018, July 14,). ICER Releases Final Evidence Report on Efficacy, Cost Effectiveness of CGRP Inhibitors for Migraine. Retrieved from https://www.ajmc.com/newsroom/icer-releases-final-evidence-report-on-efficacy-cost-effectiveness-of-cgrp-inhibitors-for-migraine

Roggenkamp, D., Kopnick, S., Stab, F., Wenck, H., Schmelz, M., & Neufang, G. (2013). Epidermal nerve fibers modulate keratinocyte growth via neuropeptide signaling in an innervated skin model. *The Journal of Investigative Dermatology, 133*(6), 1620-1628.

Rossi, P., Ambrosini, A., & Buzzi, M. G. (2005). Prodormes and predictors of migraine attack. *Functional Neurology, 20(4)*, 185-191.

Russel, F.A., King, R., Smillie, S.J., Kodji, X., & Brain, S.D. (2014). Calcitonin gene-related peptide: physiology and pathophysiology. *94(4)*, 1099-1142.

Russel, M.B., & Olesen, J. (1996). A nosographic analysis of the migraine aura in a general population. *Brain, 119(2)*, 355-361.

Russel, M.B., Rasmussen, B. K., Thorvaldsen, P., & Olesen, J. (1995). Prevalence and sex-ratio of the subtypes of migraine. *Int J Epidemiol, 24(3)*, 612-618.

Russell , M.B., Rasmussen, B.K., Thorvaldsen, P., & Olesen, J. (1995). Prevalence and sex-ratio of the subtypes of migraine. *Int J Epidemiol., 24(3)*, 612-618.

Sacco, S., & Kurth, T. (2014). Migraine and the risk for stroke and cardiovascular disease. *Current Cardiology Reports, 16*(9), 1.

Saetrum Opgaard, O., Hasbak, P., de Vries, R., Saxena, P.R., & Edvinsson, L. (2000). Positive inotropy mediated via CGRP receptors in isolated human myocardial trabeculae. *European Journal of Pharmacology, 397*(2-3), 373-382.

Sandor, P.S., Di Clemente, L., Coppola, G., Saenger , U., Fumal, A., Magis, D., Seidel, L., Agosti, R.M. & Schoenen, J. (2005). Efficacy of coenzym Q10 in migraine prophylaxis: a randomized controlled trial. *Neurology, 64(4)*, 713-715.

Saper, J.R., Lipton R.B., Kudrow, D.B., Hirman, J., Dodick, D.W., Silberstein, S., Chakhava, G. & Smith, J. (2018-2, April). *adlerbio.com.* Retrieved March 2019, from https://investor.alderbio.com/events-and-presentations: https://investor.alderbio.com/static-files/b39b7210-e43c-416b-ae8b-fddc15ac361f

Schürks, M., Diener, H.C., & Goadsby, P. (2008). Update on the prophylaxis of migraine. *Curr Treat Options Neurol., 10(1)*, 20-29.

Shi, L., Lehto, S.G., Zhu, D.X., Sun, H., Zhang, J., Smith, B.P., Immke, D.C., Wild, K.D., & Xu, C. (2016). Pharmacologic Characterization of AMG 334, a Potent and Selective Human Monoclonal Antibody against the Calcitonin Gene-Related Peptide Receptor. *J Pharmacol Exp Ther. 356(1),* 223-231

Silberstein , S.D., Holland, S., Freitag, F., Dodick, D.W., Argoff, C., & Ashman, E. (2012). Evidence-based guideline update: pharmacologic treatment for episodic migraine prevention in adults: report of the Quality Standards Subcomittee of the American Acadaemy of Neurology and the Amrican Headache Society. *Neurology, 78(17)*, 1337-1345.

Silberstein, S.D. (2000). Practice parameter: evidence-based guidelines for migraine headache (an evidence-based review):report of the Quality Standards Subcommitee of the American Academy of Neurology. *55(6)*, 754-762.

Silberstein, S. D., Dodick, D.W., Bigal, M.E., Yeung, P.P., Goadsby, P.J., Blankenbiller, T., Grozinski-Wolff, M., Yang, R., Ma, Y., & Aycardi, E.Y. (2017). Fremanezumab for the Preventive Treatment of Chronic Migraine. *N Engl J Med, 377*, 2113-2122.

Silberstein, S.D., Lipton, R.B., Dodick, D.W., Freitag, F.G., Ramadan, N., Mathew, N., Brandes, J.L., Bigal, M., Saper, J., Ascher, S., Jordan, D.M., Greenberg, S.J., & Hulihan, J. (2007). Efficacy and safety of topiramate for the treatment of chronic migraine: a randomized, double-blind, placebo-controlled trial. *Headache, 47(2)*, 170-180.

Silberstein, S., Tfelt-Hansen, P., Dodick, D.W., Limmroth, V., Lipton, R. B., Pascual, J., & Wang, S.J. (2008). Guidelines for Controlled Trials of Prophylactic Treatment of Chronic Migraine in Adults. *Cephalalgia, 28(5)*, 484-495.

Singer, N. (2010, Oct 16). Botox Shots Approved For Migraine. *The New York Times* Retrieved from https://www.nytimes.com/2010/10/16/health/16drug.html

Skljarevski, V., Matharu, M., Millen, B.A., Ossipov, M.H., Kim, B.K., & Yang , J.Y. (2018). Efficacy and safety of galcanezumab for the preventi-on of episodic migraine: Results of the EVOLVE-2 Phase 3 randomized controlled clinical trial. *38(8)*, 1442-1454.

Smillie, S. J., King, R., Kodji, X., Outzen, E., Pozsgai, G., Fernandes, E., Marshall, N., de Winter, P., Heads, R.J., Dessapt-Baradez, C., Gnudi, L., et al. (2014). An ongoing role of α-calcitonin gene-related peptide as part of a protective network against hypertension, vascular hypertrophy, and oxidative stress. *Hypertension, 63(5)*, 1056-1062.

Song, S.W., Guo, K.J., Shi, R., Cheng, Y., & Liu, Y.F. (2009). Pretreatment with calcitonin gene-related peptide attenuates hepatic ischemia/reperfusion injury in rats. *Transplantation Proceedings, 41*(5), 1493-1498.

Sparrow, A.M., & Searles, J.W. (2019). The market for migraine drugs. *Nature Reviews Drug Discovery*

Stankewitz, A., Aderian, D., Eippert, F., & May, A. (2011). Trigeminal nociceptive transmission in migraineurs predicts migraine attacks. *J Neurosci, 31(6)*, 1937-1943.

Stauffer, V.L., Dodick, D.W., Zhang, Q., Carter, J.N., Ailani, J., & Conley, R.R. (2018). 4 Evaluation of Galcanezumab for the Prevention of Episodic Migraine: The EVOLVE-1 Randomized Clinical Trial. *JAMA Neurol., 75(9)*, 1080-1088.

Stewart, W.F., Linet, M. S., Celantano, D.D., Van Natta, M., & Ziegler, D. (1991). Age- and sex-specific incidence rates of migraine with and without visual aura. *Am J Epidemiology, 134*, 1111-1120.

Taylor, F.R. (2019). CGRP, Amylin, Immunology, and Headache Medicine. *Headache, 59*(1), 131-150.

Tepper, S.J. (2018). Anti-Calcitonin Gene-Related Peptide (CGRP) Therapies: Update on a Previous Review After the American Headache Society 60th Scientific Meeting, San Fran-cisco, June 2018. *Headache, 58*, 276-290.

Tepper, S., Silberstein, S., Dodick, D., Goadsby, P., & Ailani, J. (2019, January 8). NeurologyLive launches peer exchange: 'Chronic migraine: new paradigms in management. Unprecedented Efficacy of CGRP Inhibitors for Migraine'Retrieved from https://www.neurologylive.com/peer-exchange/chronic-migraine-management/unprecedented-efficacy-of-cgrp-inhibitors-for-migraine

The Work Foundation. The socioeconomic impact of migraine. Retrieved from http://www.theworkfoundation.com/wp-content/uploads/2018/04/Society's-headache-the-socioeconomic-impact-of-migraine.-Work-Foundation.pdf

U.S Food and Drug Administration. (2018-2, October). *accessdata.fda.gov*. Retrieved March 2019, from https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/761089s000lbl.pdf

U.S Food and Drug Administration. (2018-3). *accessdata.fda.gov*. Retrieved March 2019, from https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/761063s000lbl.pdf

U.S National Library of Medicine. (2018). *clinicaltrials.gov*. Retrieved March 2019, from https://clinicaltrials.gov/ct2/show/results/NCT02066415?view=results

U.S. Food and Drug Administration. (2018). *U.S. Food and Drug Administration*. Retrieved March 2019, from https://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugInnovation/ucm592464.htm

Uddman, R., Edvinsson, L., Ekblad, E., Hakanson, R., & Sundler, F. (1986). Calcitonin gene-related peptide (CGRP): perivascular distribution and vasodilatory effects. *15(1)*, 1-23.

Vasudeva, S., Claggett, A.L., Tietjen, G.E., & McGrady, A.V. (2003). Biofeedback-assisted relaxation in migraine headache: relationship to cerebral blood flow velocity in the middle cerebral artery. *Headache, 43(3)*, 245-250.

von Luckner, A., & Riederer, F. (2018). Magnesium in Migraine Prophylaxis-Is There an Evidence-Based Rationale? A Systematic Review. *Headache, 58(2)*, 199-209.

Voss, T., Lipton, R.B., Dodick, D. W., Dupre, N., Ge, J. Y., Bachman, R., Assaid, C., & Michelson, D. (2016). A phase IIb randomized, double-blind, placebo-controlled trial of ubrogepant for the acute treatment of migraine. *Cephalalgia, 36*, 887-898.

Walter, S., Alibhoy, A., Escandon, R., & Bigal, M.E. (2014). Evaluation of cardiovascular parameters in cynomolgus monkeys following IV administration of LBR-101, a monoclonal antibody against calcitonin gene-related peptide. *mAbs, 6*(4), 871-878.

Wang, Z., Martorell, B. C., Wälchli, T., Vogel, O., Fischer, J., Born, W., & Vogel, J. (2015). Calcitonin Gene-Related Peptide (CGRP) Re-ceptors Are Important to Maintain Cerebro-vascular Reactivity in Chronic Hypertension. *PLoS One, 10(4)*, e0123697.

Wang, L.H., Zhou, S.X., Li, R.C., Zheng, L.R., Zhu, J.H., Hu, S.J., & Sun, Y.L. (2012). Serum levels of calcitonin gene-related peptide and substance P are decreased in patients with diabetes mellitus and coronary artery disease. *The Journal of International Medical Research, 40*(1), 134-140.

Weiller, C., May, A., Limmroth, V., Jüptner, M., Kaube, H., Schayck, R.V., Coenen, H.H., & Diener, H.C. (1995). Brain stem activation in spontaneous hu-man migraine attacks. *Nat Med., 1(7)*, 658-660.

Winegarden Wayne. (2018, April). The ICER Myth. Retrieved from https://allianceforpatientaccess.org/press-release-new-paper-the-icer-myth-explores-drawbacks-of-icers-evidence-reports-and-their-impact-on-patients/

Yadav, S., Yadav, Y.S., Goel, M.M., Singh, U., Natu, S.M., & Negi, M.P. (2014). Calcitonin gene- and parathyroid hormone-related peptides in normotensive and preeclamptic pregnancies: a nested case-control study. *Archives of Gynecology and Obstetrics, 290*(5), 897-903.

Zeller, J., Poulsen, K.T., Sutton, J.E., Abdiche, Y.N., Collier, S., Chopra, R., Garcia, C.A., Pons, J., Rosenthal, A., & Shelton, D.L. (2008). CGRP function-blocking antibodies inhibit neurogenic vasodilatation without affecting heart rate or arterial blood pressure in the rat. *British Journal of Pharmacology, 155*(7), 1093-1103.

Zhang, F., Wang, S., Signore, A.P., & Chen, J. (2007). Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia. *Stroke, 38*(8), 2329-2336.

Zhang, J. Y., Yan, G. T., Liao, J., Deng, Z. H., Xue, H., Wang, L. H., & Zhang, K. (2011). Leptin attenuates cerebral ischemia reperfusion injury partially by CGRP expression. *European Journal of Pharmacology, 671*(1-3), 61-69.