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Antoinette Maassen van den Brink
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Monoclonal Antibodies in Headache

From Bench to Patient

Springer

Headache

Series Editor

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Roma, Italy

The purpose of this Series, endorsed by the European Headache Federation (EHF), is to describe in detail all aspects of headache disorders that are of importance in primary care and the hospital setting, including pathophysiology, diagnosis, management, comorbidities, and issues in particular patient groups. A key feature of the Series is its multidisciplinary approach, and it will have wide appeal to internists, rheumatologists, neurologists, pain doctors, general practitioners, primary care givers, and pediatricians. Readers will find that the Series assists not only in understanding, recognizing, and treating the primary headache disorders, but also in identifying the potentially dangerous underlying causes of secondary headache disorders and avoiding mismanagement and overuse of medications for acute headache, which are major risk factors for disease aggravation. Each volume is designed to meet the needs of both more experienced professionals and medical students, residents, and trainees.

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Monoclonal Antibodies in Headache

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ISSN 2197-652X ISSN 2197-6538 (electronic)
Headache
ISBN 978-3-030-69031-1 ISBN 978-3-030-69032-8 (eBook)
https://doi.org/10.1007/978-3-030-69032-8

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Foreword

This volume, the 13th in the Headache series, continues to be endorsed by the European Headache Federation, and covers a topic currently at the center of the clinical-scientific interest of *headache medicine:* the migraine therapy with monoclonal antibodies to calcitonin gene-related peptide or its receptor.

This new pharmacological class, according to the increasing number of real-world evidence studies, is fulfilling its promise to be *a disease-modifying migraine drug,* with a very high efficacy rate, an excellent safety profile, and an acceptable non-responders rate. Its effectiveness is shown very quickly, so it deserves the definition of "drugs without the taxiing phase." Its fields of application, beyond migraine and cluster headache, are under continuous study, and possible expansions are undergoing due diligence. A new era for migraine has begun, and this volume describes every scientific and clinical angle of it in details.

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Preface

When a year ago we decided to involve distinguished experts in the project of this volume, we could only imagine how their clinical application would revolutionize the therapeutic area of headaches. In fact, the appearance of monoclonal antibodies for calcitonin gene-related peptide or its receptor (CGRP (r)) today represents a radical change in migraine prophylaxis therapy and a new gold standard in the level of satisfaction reported by patients. Even with an acceptable percentage of non-responders, these drugs, erenumab, galcanezumab, fremanezumab, and eptinezumab, now and in the near future represent the cornerstone for the treatment of patients with high frequency or chronic migraine, or refractory migraine attacks resistant to previous preventive therapies. The benefit they find in this therapy is immediate and is accompanied by an excellent tolerability profile. Further tests will come from real-world studies, especially on the cardiovascular safety in migraine patients.

This volume explores all aspects of these molecules, describing their pharmacokinetics, pharmacodynamics, randomized controlled studies, and the most recent real-world evidence studies. Future applications of this pharmacological class in forms of headache other than migraine are also analyzed.

This volume is dedicated both to clinicians who need guidance in the therapeutic orientation of migraine prevention, as well as to residents of Neurology, Internal Medicine, Pharmacology, and PhD students, and to students of the Faculty of Medicine.

Rotterdam, The Netherlands — Antoinette Maassen van den Brink
Rome, Italy — Paolo Martelletti

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Chapter 1
The CGRP Family of Neuropeptides and their Receptors in the Trigeminovascular System

Lars Edvinsson and Karin Warfvinge

1.1 Introduction

Primary headache disorders affect more than 1 billion of people and together they represent the leading cause of years lived with disability worldwide, with a considerable unmet need for diagnosis and therapy. The International Headache Society (IHS) has provided the third edition of the diagnostic criteria ICHD-3 [1] and recently added the details for classification of facial pain [2] which collectively covers basically all the sensory systems of the head. Common to all these is the trigeminal system [3] and with this in mind we envision that different syndromes related to the trigeminal system might be treated with drugs related to the calcitonin gene-related peptide (CGRP) family of peptides since this is the system most densely expressed in the trigeminal ganglion (TG).

Migraine therapy has in the past relied on medications with different sites of action; their use has been hampered by low efficacy and heavy burden of side effects [4]. During the last decades, insights into the molecular mechanisms have provided novel and specific targets: triptans, gepants, and ditans for acute therapy, and monoclonal antibodies acting on the CGRP system and gepants for prophylaxis [5].

The TG gives off peripherally the ophthalmic, the maxillary, and the mandibular nerves, and connects centrally to the trigeminal nucleus caudalis (TNC) and dorsal roots C_1–C_3 [6] to form the trigeminovascular system, which putatively is the main site of action of these new therapies. The TG is the main sensory ganglion for cranial structures, both intracranial and extracranial. The neurons within the TG are firmly enveloped by satellite glial cells (SGCs), demonstrating the possibility for a

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_1

close interaction between the neurons and glial cells, coupled to each other by gap junctions [7]. In addition, CGRP is richly located at numerous sites throughout the central and peripheral nervous systems [8, 9]. A sizable population of CGRP neurons within the TG (almost 50%) signifies a major role for CGRP in trigeminal transmission. This has been verified by specific immunohistochemical staining with CGRP antibodies and in situ hybridization to localize cellular mRNA for CGRP. The CGRP-positive neurons give off fibers, which are un-myelinated and small–medium in diameter, which is indicative of cell bodies of the C-type of sensory pain fibers. Research on primary headaches has revealed an important role of CGRP, mainly related to the head pain, while the involvement and expression of other members of this family of peptides are less well known [5].

Here we provide an overview of the CGRP family of peptides, sharing structural homology with CGRP, and these are calcitonin (CT), adrenomedullin (AM), and amylin (AMY). They have a widespread distribution throughout the body, with particular abundance in the brain, the gastrointestinal system, and in various parts of the circulation [10]. The members of the CGRP family might hypothetically be clinically relevant drug targets due to their role in the regulation of several critical homeostatic processes elsewhere in the body [11].

1.2 The Receptors of the CGRP Family

The peptides are ligands for a closely related family of G protein-coupled receptors (GPCRs), a shared structural homology receptor system which is complicated [12]. Receptors are formed from two GPCRs, the calcitonin receptor-like receptor (CLR) and the calcitonin receptor (CTR), which interact with receptor activity-modifying protein (RAMPs) to form heterodimers. The RAMPs are a small family of three proteins (RAMP 1–3) that are single transmembrane-spanning proteins (TM), which may modify the pharmacology, functionality, and cell trafficking of the specific GPCRs [13]. The currently most central is the seven transmembrane (7TM) complex—CLR—which is a required element of receptors for CGRP and adrenomedullin (AM_1 and AM_2). Early studies showed that transfecting cells with only CLR revealed no response to CGRP [14, 15]. It was only after the demonstration of the fusion of RAMP1 to CLR that resulted in the formation of a functional receptor to CGRP [16]. Amylin receptors are formed by the CT receptor (CTR) and are associated with the RAMPs. CTR can also act as a calcitonin receptor by itself (without a RAMP). The amylin receptors are formed by CTR that is heterodimerized with a RAMP to form AMY receptors [17]. Due to the complexity of this peptide–receptor system, their expression in the trigeminal system is unclear and their functional roles yet to be resolved [18]. To summarize the CGRP family of receptors: CGRP receptor (CLR/RAMP1), AM_1 receptor (CLR/RAMP2), AM_2 receptor (CLR/RAMP3), CT receptor (CTR), AMY_1 receptor (CTR/RAMP1), AMY_3 receptor (CTR/RAMP3).

1.3 The CGRP Family of Ligands and their Distribution

The first study of CGRP distribution in the trigeminal ganglion was performed in the cat in 1985 [19] and it was followed by numerous studies on CGRP distribution in different parts of the body. In the last decade, work has in detail described CGRP and its receptor components in the TG system of rat, monkey, and man [8, 20, 21] and in the rat retina [22].

CGRP is expressed in a granular pattern in small- to medium-sized TG neurons [8, 23, 24] where CGRP is packed in vesicles that are surrounded by the Golgi apparatus (Fig. 1.1a). In addition, pearl-like CGRP immunoreactivity is detected in fibers that are of the C-type of sensory unmyelinated nerves (Fig. 1.1e). The myelinated fibers do not contain CGRP despite some previous work, putatively due to the use of poor antibodies [24]. The receptor components, CLR and RAMP1, are expressed in neurons (mainly the larger ones), seen also in SGCs and the thick fibers, typical for Aδ-fibers (Fig. 1.2a, b). CGRP, CLR, and RAMP1 distribution in rat TG has been examined in detail [8, 23, 24]. This has today been confirmed by many researchers and resulted in the successful development of both monoclonal antibodies for migraine prophylaxis and small molecules towards the CGRP receptor (gepants) [5].

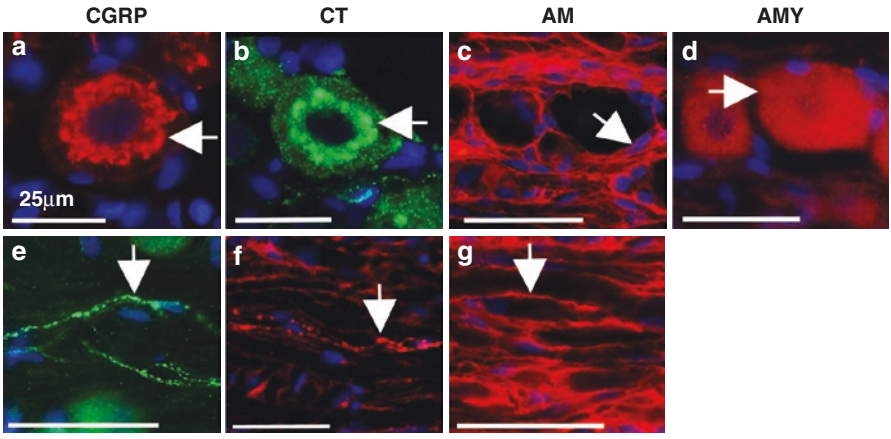

Fig. 1.1 Ligand immunohistochemistry. (**a**) CGRP is expressed in a granular pattern in many neurons (arrow), mainly in small- to medium-sized neurons (arrowheads). The cellular CGRP is packed in vesicles that are surrounded by the Golgi apparatus. (**b**) CT immunoreactivity displayed a similar pattern as for CGRP; granular staining of small- to medium-sized neurons (arrow). (**c**) AM was expressed in the glial cells, both the SGCs (arrow). (**d**) AMY was exclusively expressed in the neurons, mainly small to medium sized. In some of the cells, the expression was granular, but in others a general cytoplasmic immunoreactivity (arrow). (**e**) In addition to CGRP cell soma immunoreactivity, pearl-like CGRP expression was detected in fibers that are of the C-type of sensory unmyelinated nerves (arrow). The myelinated fibers do not contain CGRP. (**f**) In CT immunohistochemistry, pearl-like staining of fibers was found (arrow). (**g**) AM immunohistochemistry disclosed positive cells enveloping the neuronal processes (arrow), probably myelinating cells

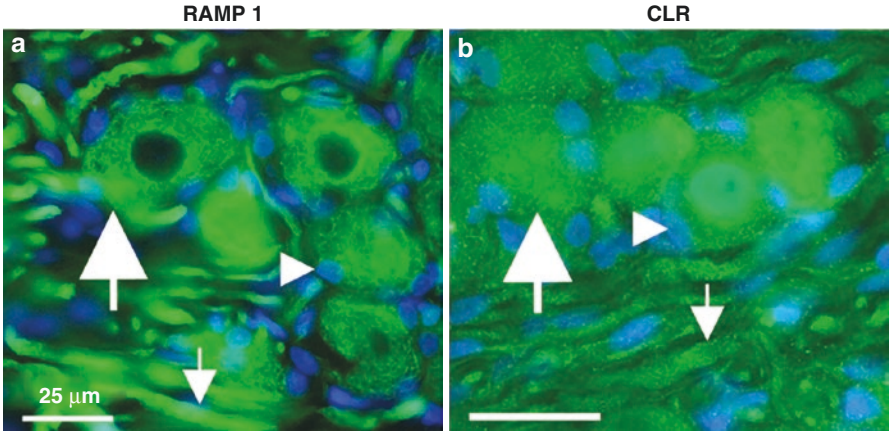

Fig. 1.2 Receptor immunohistochemistry. (**a**, **b**) The CGRP receptor components, RAMP1 and CLR, were expressed in neurons (mainly the larger ones, thick arrows), in the SGCs (arrowheads), and the thick fibers, typical for Aδ-fibers (thin arrows)

CT was first discovered more than 50 years ago [25] and is a hormone produced by C cells in the thyroid gland: the main role is to reduce plasma calcium and to promote bone formation [26]. CT is used clinically in the treatment of bone disorders characterized by increased bone resorption, osteoporosis, and hypercalcemia due to malignancy, with some pain relief [26]. However, there is still yet much to learn about the action and role of CT related to migraine/headaches.

The presence of CT and its receptor in a large number of different cell types and tissues suggests multiple physiological roles [26]. Binding of CT to a specific receptor induces morphological changes in osteoclasts, which results in bone resorption [27]. CT has not been shown to be expressed in the nervous system; however, binding sites for CT are found in many brain structures [11]. Recently we reported that the CT expression occurs in a similar pattern as for CGRP in the trigeminal system; CT is packed in vesicles that are surrounded by the endoplasmic reticulum/Golgi apparatus and pearl-like CT immunoreactivity is seen in the C-type of sensory fibers (Fig. 1.1b, f) [18]. In fact, there is co-expression of CGRP and CT in small- to medium-sized TG neurons. The CT immunoreactivity displayed a similar pattern as was observed for CGRP; a granular staining of small- to medium-sized neurons and pearl-like staining of fibers in the TG. Also, many of the SGCs are CT immunoreactive [18]. This might indicate that the C-fibers might contain CT in addition to CGRP; hence a role in pain disorders might be hypothesized.

AMY has an affinity for CGRP receptors and CGRP for AMY_1 receptors [11]. AMY and CGRP are the most closely related peptides in terms of amino acid sequence which may cause an overlap in the ability to activate each receptor. Human AMY was probably first observed in 1901, described as hyaline deposits in the

pancreas of patients with type 2 diabetes [28]. AMY was first isolated in 1987 [29] and is an endocrine hormone that signals to the brain and acts as a satiety factor. AMY may also have other roles [30]. Research on AMY deposition in brain neurons has mainly been in the focus on a role for AMY in Alzheimer's disease [31]. Recently, it was shown that AMY may alter human brain pericytes viability [32]. In addition, AMY immunoreactivity has been described in several places along the gut and in some neurons [30, 33]. We have reported early on AMY expression in the TG of cat and that AMY can dilate in vitro as well as in vivo cerebral vessels [34].

The receptors for CGRP and AMY are related and share components, CLR/RAMP1 (ligand CGRP) and CTR/RAMP1 (ligand AMY). Given the close relationship between AMY and CGRP, especially that they share the AMY_1 receptor, it is worthwhile to consider whether amylin can trigger a migraine. Consequently, using the infusion model AMY was reported to induce migraine-like attacks, which support the development of selective monoclonal antibodies toward AMY (AMGEN, Elli Lilly Pharma). The results from a trial on migraine patients did however not reveal any prophylactic effect [35]. We are still awaiting the full report.

Was there any hint of this result from our studies? AMY was exclusively expressed in a small number of neurons, mainly small to medium sized. In some of the cells, the expression was granular, but in others a general cytoplasmic immunoreactivity was observed (Fig. 1.1d). The location resembles that of CGRP, but the low degree of expression may hint that the effect in a clinical study would be low.

AM is expressed in the thin cytoplasm of the glial cells, both the SGCs and cells enveloping the neuronal processes, probably myelinating cells (Fig. 1.1c, g). In addition, immunoreactivity is found in blood vessel walls, indicating vascular endothelial staining. Historically, AM is chiefly found in endothelial cells and was first isolated in 1993 [36]. AM has been found to be generally expressed and participate in a variety of physiological functions in the body including vasodilation, bronchodilatation, growth, and hormone regulation [37]. Furthermore, AM is involved in several pathophysiological processes such as hypertension, retinopathy, and tumor genesis [38].

In 1994, active production of AM was demonstrated in cultured endothelial cells [39]. In mammals, endothelial AM immunoreactivity has been inconsistently reported, the reason for this might be that AM is present in low concentrations in the vascular endothelium [40]. AM has also been localized in neurons and glial cells [41]. AM has been discussed to have a role in migraine due to its close molecular relation to CGRP [42]. However, using the infusion method [43] AM did not cause migraine-like attacks, consequently, the idea was abandoned [44]. This finding is supported by a recent study of the TG system, showing that AM was not observed in neurons but only in glial cells, SGCs, and cells enveloping the neuronal processes, probably myelinating cells [18]. Experimental studies on the cranial circulation showed vasodilatation which correlates with the demonstration of AM immunoreactivity in vascular endothelium.

1.4 Relation Between Expression of Ligands and Receptors

1.4.1 Ligand Experiments

In order to provide morphological clues for functional relations among the different peptides and receptors of the CGRP family within the trigeminal system, various combinations of immunohistochemical staining with different combinations of antibodies have recently been performed [18]. First, double staining with CGRP and CT antibodies showed co-expression in the small- to medium-sized neurons. However, it was in addition observed that CT was expressed in the SGCs. This suggests that CT might have a role in trigeminal function. CGRP and AMY double staining showed co-expression in some of the small- to medium-sized neurons and thin C-fibers, however, some were seen to only express CGRP or AMY. The number of CGRP positive cells appeared to be far more abundant than those storing AMY.

1.4.2 Receptor Expressions

RAMP1 and CLR are co-expressed mainly in larger neurons and the thick myelinated neuronal fibers [8, 45]. We observed the CGRP receptor at many places like distally in the dura mater, associated both with the vessels and at avascular sites. In larger arteries, these fibers run in the adventitia, close to the C-fibers. Quite interestingly, the C-fibers storing CGRP came in close apposition to the nodes of Ranvier in the Aδ-fibers when we examined the trigeminal nerves in more detail [45]. This led to the suggestion that at the nodes of Ranvier local release of CGRP may act on CGRP receptors on the Aδ-fibers to modify the signaling and perhaps the pain perception. In addition, in this region, CLR/RAMP1 as well as a complete CGRP receptor antibody immunoreactivity were observed on the Aδ-fibers and associated with protein kinase A (cAMP stimulant), which provides a link toward interaction between the two main fiber types of the trigeminal system [45]. As discussed, this is a putative site for gepants and monoclonal antibodies to interact with the trigeminal system and the pain perception.

RAMP1 was expressed in the cytoplasm of neurons and SGCs and in the thick myelinated Aδ-fibers (Fig. 1.2a). The CTR was expressed with varying intensity in the cytoplasm of most neurons and in SGCs in the TG (Fig. 1.3a). RAMP1 and CTR double immunohistochemistry showed co-expression in some neurons, SGCs, and fibers, indicative of the presence of the AMY_1 receptor expression.

RAMP2 is exclusively expressed in the nuclei, both neuronal and glial cell nuclei (Fig. 1.3b).

RAMP3 and CTR double immunohistochemistry revealed a possible AMY_3 receptor in the SGCs.

RAMP3 is expressed in the nuclei, both neuronal and glia cell nuclei, and in addition in some SGCs (Fig. 1.3c). CLR is expressed in neurons, in the SGCs, and

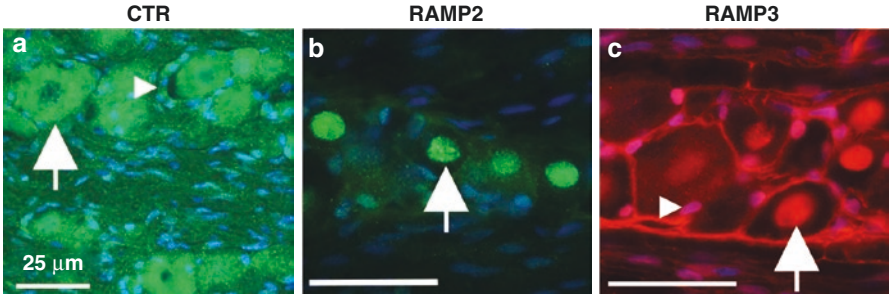

Fig. 1.3 Receptor immunohistochemistry. (**a**) CTR was expressed in varying intensity in most neurons (thick arrow) and SGCs (arrowheads). (**b**, **c**) RAMP2 and RAMP3 were expressed in the nuclei (arrows). In addition, RAMP3 was expressed in the glial cells (arrowhead)

the thick fibers. Double immunohistochemistry showed co-expression in the SGCs, which suggests a presence of AM_2 receptor.

1.4.3 Ligand/Receptor Expression

The ligands are expressed in different manners: CGRP and CT are expressed in many neurons and pearl-like unmyelinated C-fibers (Fig. 1.1a, b). In addition, SGCs are CT immunoreactive. AMY was exclusively expressed in some of the neuron (Fig. 1.1d). AM was expressed in the glial cells (Fig. 1.1c, g).

The receptor components show similar diversity in expression: CLR and RAMP1 immunoreactivity occur in the neurons, in the SGCs, and the thick Aδ-fibers (Fig. 1.2a, b), RAMP2/3 occur in nuclei (Fig. 1.3b, c) and CTR in most neurons and SGCs (Fig. 1.3a).

We have previously shown that there is no co-localization between CGRP and CLR/RAMP1 [8]. In contrast, the double CT and CTR immunohistochemistry showed that all CT immunoreactive cells could also be CTR positive [18]. No co-localization was found in the fibers (CT positive) or the glial cell nuclei (CTR positive). CT and RAMP1 double staining revealed co-localization in some neurons. However, some neurons were only CT immunoreactive and some only RAMP1 immunoreactive. The same pattern of immunoreactivity was found using AMY and RAMP1 antibodies; some neurons showed co-localization, some AMY, and some RAMP1 immunoreactivity.

AM is expressed in the glial cells and RAMP2 in the nuclei, consequently no co-localization was found using double immunohistochemistry.

To summarize, the CGRP receptor (CLR/RAMP1) is mainly expressed in the large neurons and SGCs, the AM_2 receptor (CLR/RAMP3) in some SGCs, the AMY_1 receptor (CTR/RAMP1) in large neurons and SGCs, while the AMY_3 receptor (CTR/RAMP3) was observed in some SGCs.

How Are the CGRP Family of Peptides and their Receptors Related and Involved in the Trigeminal System Function?

There are today in principle two ways to explain migraine pathophysiology, but both regard the trigeminal system as a key part at least in the headache phase. Firstly, the trigeminal system provides the link between the peripheral primary afferents and the central terminals of the trigeminal nucleus caudalis. Activation of this pathway may result in sensitization within second-order neurons and drive the CNS aspects of the migraine attacks [46]. Secondly, elegant studies by May and colleagues have demonstrated that activation of the hypothalamus is the early (even prodromal) site where the migraine attack starts [47, 48]. Connectivity studies have revealed that other CNS regions are subsequently activated including the brainstem from which links are available to activate or modulate the trigeminal system function [46]. In both hypotheses, the trigeminal system plays a key role and the currently available monoclonal antibodies toward CGRP and the CGRP receptor are effective [5] despite their inability to penetrate the blood–brain barrier [49, 50].

Each of the peptides of the CGRP family exhibits a distinct selection of biological actions [17]. CGRP and AMY are the most closely related peptides in terms of amino acid sequence which may cause an overlap in their ability to activate each receptor. Both CGRP and AMY are reported to have effects relating to pain, though there are still limited data and it is unclear how much overlap there is because the peptides are not usually tested in the same study [51]. Also, the receptors themselves are related and share components. The relative potency of CGRP, CT, AMY, or AM to the different receptor complexes is a complicated topic. This makes the work challenging because of the cross-reactivity [11, 16]. In addition, the release of a peptide will result in a very high concentration just at the receptor site, while circulating levels vary considerably. The study of the nodes of Ranvier in the trigeminal system demonstrated CGRP containing boutons with CGRP containing vesicles in C-fibers may directly release CGRP to reach the adjacent Aδ-fibers with CGRP receptors linked to the formation of cAMP and protein kinase A [45]. This mechanism may alter the signaling in the Aδ-fibers and contribute to the pain signaling.

Previously, the main focus has been on studies of CGRP and its receptor, though this is a verified successful story in science [5], recent work on the other ligands and receptors of the CGRP family of peptides points toward other possible targets putatively able to modify the function of the trigeminal system [18].

AMY has before been demonstrated in rat, cat, and man trigeminal system [34, 52]. To our knowledge, immunohistochemistry using RAMP2 and RAMP3 antibodies has hitherto not been demonstrated in the CNS [18]. It is important to compare the peptides side-by-side in order to understand which receptor(s) that may have the potential to be new antimigraine targets.

The CGRP family of peptides is ligands for closely related family B of GPCRs and share structural homology [12]. Class B GPCRs are involved in major biological and pathophysiological functions [53]. It is now clear that RAMPs can interact with a wider range of GPCRs; all three RAMPs can interact with the $VPAC_1$ (vasoactive intestinal polypeptide/pituitary adenylate cyclase-activating peptide)

receptor. In addition, RAMPs can produce a number of different effects on ligand binding, signal transduction, and receptor trafficking [54]. Thus, the field is expanding.

Within the CGRP family of peptides, the N-terminus and C-terminus are the most highly conserved regions with more divergence in the midportion of each peptide, suggesting an importance in the retention of the N-terminal and C-terminal for biological activity [55]. As to the receptor components, CTR was cloned in 1991 [56], CLR in 1993 [57], and RAMP1 was discovered in 1998 [15]. A sequence database search for expressed sequences like RAMP1 identified RAMP2 and RAMP3 [58].

We performed double immunohistochemistry with ligand to ligand and ligand to receptor. No co-localization was found between AM and the other members of the CGRP family, even though overlap is known to occur because the peptides share features. The AM receptors consist of CLR and RAMP2 (AM_1 receptor) and CLR and RAMP3 (AM_2 receptor). Double immunohistochemistry with AM and RAMP2 (AM receptor component) antibodies showed no co-localization; AM is mainly expressed in the glial cells and RAMP2 in the nuclei, consequently no co-localization was observed. Receptor–receptor immunoreactivity with RAMP3 and CLR showed weak double staining in the glial cells, mainly SGCs. However, relatively low expression does not automatically translate into little function.

1.5 Conclusion

It is important to compare peptides and receptors side-by-side in studies to help address questions of actions resulting from cross-reactivity between receptors. From this, we conclude that the AM_2, AMY_1, and AMY_3 receptors occur in rat TG but mainly in the SGCs.

Several of the diverse biological actions of the CGRP family of peptides are clinically relevant. The demonstration of the specific ligand and receptor sites in neurons of the rat trigeminal ganglion highlights the importance of CGRP and the CGRP receptor as a viable mechanism in primary headache disorders. Understanding the location and distribution is important in deciding on how to identify future targets for antimigraine medications and this information will facilitate future drug developments.

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Chapter 2
Pharmacology; Where Do the mAbs Act, Gepants Versus mAbs

Alejandro Labastida-Ramírez

2.1 Pharmacology

Multiple lines of evidence have implicated calcitonin gene-related peptide (CGRP) as a key neuropeptide involved in the pathophysiology of migraine headache [1–3], turning CGRP and its receptor into attractive targets for the development of novel pharmacological treatments for this debilitating condition. Remarkably, all migraine clinical trials with anti-CGRP drugs, either for prophylactic or acute treatment, so far have been positive [4, 5].

Two homolog isoforms have been described, α-CGRP and β-CGRP [6]. α-CGRP is highly expressed throughout the trigeminovascular nociceptive system, predominantly in sensory Aδ- and C-fibers arising from the trigeminal ganglion [7, 8], and as the majority of headache-related studies focus only on α-CGRP, it will in this chapter simply be referred as CGRP unless stated otherwise.

This neuropeptide is involved in diverse biological processes, and its somatosensory functions include heat perception and itch, as well as modulation of craniofacial nociception and sensitization of trigeminal afferents from meningeal nociceptors, which are relevant in migraine pathophysiology [9, 10]. Since it has been shown that CGRP is released from trigeminal sensory fibers in significant amounts not only during migraine but also during cluster headache attacks [1, 11], novel pathophysiologically driven treatments were developed to target CGRP signaling through either inhibition of the release or direct blockade of CGRP or its receptor.

Initially, selective small-molecule CGRP receptor antagonists (Gepants) were synthesized for binding the CGRP receptor, blocking its action by preventing binding of the CGRP peptide. This was followed by the development of anti-CGRP monoclonal antibodies (mAbs), also designed to directly bind to the receptor but

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_2

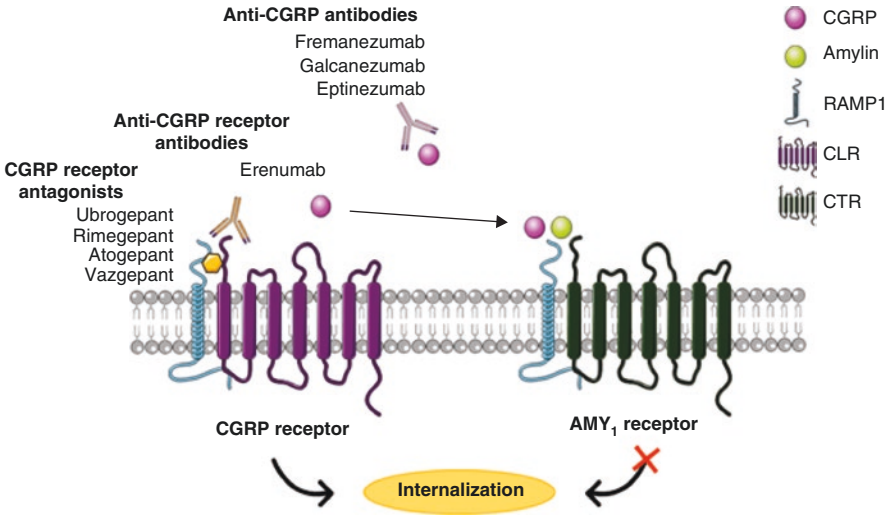

Fig. 2.1 CGRP and amylin 1 (AMY_1) receptors are formed by the association of either CLR or CTR with RAMP1, respectively. CGRP and amylin are equipotent at the AMY_1 receptor, while CGRP is more potent at the canonical CGRP receptor. These receptors have a distinct internalization profile. Current antimigraine drugs targeting CGRP or its receptor are shown. Modified from [18], published under CC BY 4.0 and from [17]

also to the peptide, both preventing CGRP signaling [12, 13]. As shown in Fig. 2.1, in contrast to the classic G-protein-coupled receptor, the functionality of the canonical CGRP receptor complex depends on the presence of its two components: a receptor activity-modifying protein 1 (RAMP1) and a G-protein-coupled calcitonin receptor-like receptor (CLR) [14]. Moreover, it has been shown that CGRP is equipotent at activating a second CGRP responsive receptor, the amylin 1 receptor (AMY_1), which also contains RAMP1 but is coupled to the calcitonin receptor (CTR) [15, 16]. Activation of either receptor results in the accumulation of cAMP [17].

Amylin and CGRP are the most closely related peptides of the CGRP family in terms of amino acid sequence and function [19]. Both peptides have been shown to co-localize in trigeminal nociceptors [20], and knockout mice of either CGRP or amylin displayed reduced pain responses [21, 22]. Moreover, current experimental data suggest that there is an important possibility for cross-reactivity between these peptides and its receptors [17, 20], and as previously suggested by MaassenVanDenBrink et al. [23] this opens the possibility of several scenarios: (i) after CGRP is scavenged by a CGRP antibody, other peptides with high affinity for the CGRP receptor (i.e., amylin) may bind to this receptors; (ii) AMY_1 receptor signaling, either through amylin or CGRP, compensates CGRP receptor blockade; and (iii) some anti-CGRP drugs indirectly antagonize the AMY_1 receptor. If so, antagonists of the AMY_1 receptor could represent a new antiheadache target. In support of this, a new study revealed that after repeated agonist stimulation, the AMY_1

receptor undergoes scarce internalization in comparison to the CGRP receptor, suggesting that during a migraine or cluster headache attack where CGRP levels are markedly increased, indirect activation of the AMY_1 receptor could contribute to sustained dysfunctional nociception [17]. However, the (patho)physiological role of amylin in the trigeminovascular system has not been completely understood, and it remains to be determined whether one or both receptors underlie CGRP actions in headache and to what extent the novel anti-CGRP drugs interact with the AMY_1 receptor.

As we are starting to understand the complexity of the CGRPergic system and the interactions with its family of peptides, a fundamental question remains controversial and highly debated: where is the precise antimigraine site of action (peripheral vs. central) of the anti-CGRP drugs? Therefore, the following sections will provide a critical summary of the current understating of the plausible site of action of CGRP antagonists and antibodies.

2.2 Gepants

Due to the unquestionable role of CGRP in migraine headache pathophysiology, selective small-molecule CGRP receptor antagonists, such as olcegepant and telcagepant, were synthesized and proved to be effective in the acute treatment of migraine [24, 25]. Although the first generation of gepants was promising, pharmacokinetic and hepatotoxicity limitations halted their development (reviewed in [13]), nevertheless, four new gepants have been developed for either the acute or prophylactic treatment of migraine, and all have shown efficacy in randomized clinical trials (see Table 2.1). This new generation includes three orally bioavailable drugs (ubrogepant, rimegepant, and atogepant) and zavegepant, the first intranasal gepant which could also be administered subcutaneously or orally [26].

Despite the earlier initiation of gepants drug discovery efforts and clinical trials [27], mAbs were approved first and are currently available for the prophylactic treatment of migraine. Though, due to gepants early synthesis and development, considerably there are more preclinical studies that have addressed their antimigraine site of action.

These drugs are capable of crossing the blood–brain barrier (BBB) based on their small molecular weight (<0.9 kDa, [13]); hence, it would be expected that their antimigraine efficacy is the result of antagonizing the CGRP receptor both centrally and peripherally. In this sense, electrophysiological studies in rats have revealed that the central structures of the cranial nociceptive systems being targeted by gepants include second- and third-order nociceptive trigeminovascular neurons [28, 29], but also descending pain modulatory systems such as the periaqueductal gray and nucleus raphe magnus [30, 31]. However, positron emission tomography (PET) studies with the CGRP receptor tracer [^{11}C]MK-4322 seem contradictory, as they suggest that gepants do not require to penetrate the BBB to exert their antimigraine

Table 2.1 Summary of small molecule antagonists (gepants) and monoclonal antibodies (mAbs) targeting CGRP or its receptor (CLR/RAMP1). Taken and modified from [13]

Drug	Target	Route of administration	Dose	Approved migraine treatment/ (under RCTs)	Additional off-label (non-migraine) investigations
Gepants					
Ubrogepant (Ubrelvy®)	CLR/ RAMP1	Oral	50 or 100 mg	Acute	
Rimegepant (Nurtec®)	CLR/ RAMP1	Oral	75 mg	Acute (prophylactic)	Refractory trigeminal neuralgia [35]
Atogepant	CLR/ RAMP1	Oral	10, 30 or 60 mg QD	(Prophylactic)	
Zavegepant	CLR/ RAMP1	Intranasal	10 or 20 mg	(acute, prophylactic)	Anti-inflammatory (COVID-19) [36]
mAbs					
Erenumab (Aimovig®)	CLR/ RAMP1	Subcutaneous	70 mg or 140 mg QM	Prophylactic	Hemicrania continua, medication overuse headache, persistent PTH; facial pain, rosacea [37–41]
Fremanezumab (Ajovy®)	CGRP ligand	Subcutaneous	225 mg QM or 675 mg QTLY	Prophylactic	CADASIL, PTH; fibromyalgia, interstitial cystitis–bladder pain syndrome [42–45]
Galcanezumab (Emgality®)	CGRP ligand	Subcutaneous	240 mg LD followed by 120 mg QM 300 mg LD followed by 300 mg QM (eCH)	Prophylactic	Vestibular migraine [46]
Eptinezumab (Vyepti®)	CGRP ligand	Intravenous	100 mg or 300 mg QTLY	Prophylactic (acute-RELIEF trial [47])	

eCH Episodic cluster headache, *LD* Loading dose, *PTH* Post-traumatic headache, *QD* Once daily, *QM* Once monthly, *QTLY* Quarterly, *RCTs* Randomized-controlled trials

action. In brief, Sur et al. [32] found in nonhuman primates that after the oral administration of two gepants (telcagepant and MK3207), only a small percentage could be detected in cerebrospinal fluid (CSF) as compared to plasma (CSF/plasma ratio of ~1% and ~3%, respectively). Later, Hostetler et al. [33] showed that only supratherapeutic doses of telcagepant were able to achieve a moderate CGRP receptor occupancy in primate and human brain regions (43–58%), whereas a clinically

relevant dose in healthy volunteers only achieved low receptor occupancy (≤10%). Finally, another PET study with the same tracer found no evidence of CGRP receptor central occupancy after therapeutic doses of telcagepant in migraine patients during ictal and interictal periods [34]. Thus, collectively, these results indicate that at therapeutic concentrations, a central antagonism of the CGRP receptor is not required for gepants' efficacy in migraine treatment. However, the spatial resolution of these imaging studies does not exclude a central site of action at circumventricular organs (areas close related to the CSF not covered by the BBB), so it remains to be determined experimentally whether gepants target these areas, or whether some patients also require central antagonism of the CGRP receptor to achieve migraine relief, as have previously been suggested [13, 33].

The peripheral antimigraine sites of action of the gepants are discussed in the following anti-CGRP antibody section.

2.3 mAbs Targeting the CGRPergic System

In the last decades, the use of mAbs has emerged as a major class of therapeutic agents for oncological and immunological diseases and these are recently expanding into other medical areas, including the headache field. The use of mAbs has several advantages such as high affinity and selectivity for their molecular targets, long-circulating plasma half-lives facilitating adherence to treatment, and limited potential for nonspecific hepatic and renal toxicity, as they are exclusively metabolized by the reticuloendothelial system (reviewed in [48, 49]. This pharmacological profile leads to few off-target (side) effects and drug–drug interactions, making mAbs an attractive alternative to traditional small molecule therapies, especially for the prophylactic treatment of migraine. However, these drugs have poor oral bioavailability (<1%) given their large molecular weight, polarity, and restricted membrane permeability, as well as limited gastrointestinal stability [48]. Thus, mAbs can only be administered parenterally, mostly by subcutaneous, intramuscular, or intravenous injections, the subcutaneous route being the most widely used due to convenience and the possibility of patient self-administration [48]. Importantly, as mentioned by Charles et al. [49], in case a severe adverse event occurs, no noninvasive method to rapidly remove the drug exists yet, which could limit the use of these drugs and favor the prescription of gepants.

As shown in Table 2.1, there are currently four immunoglobulin G-derived mAbs approved for migraine prophylaxis: one fully human mAb targeting the CGRP receptor (erenumab) and three humanized mAbs targeting CGRP itself (fremanezumab, galcanezumab, and eptinezumab). Despite the differences in targets, dose regimens, and production (humanized vs human), the four drugs have shown remarkably similar efficacy, tolerability, and limited adverse side effects in randomized control trials of migraine, either episodic (<15 headache days/month) or chronic (≥15 headache days/month) [27, 50].

At this time, galcanezumab is the only anti-CGRP mAb approved by the FDA for the treatment of episodic cluster headache. Moreover, some of these drugs are currently being researched for the treatment of other primary (including acute migraine treatment) and secondary headaches, as well as non-cranial pain conditions (see Table 2.1), highlighting the important role of CGRP as neuromodulator of peripheral and central sensory neurons. In general, these antibodies act as scavengers of circulating CGRP molecules or blockers of the CGRP receptor [13, 51]; however, their exact site of action has not been completely elucidated.

In contrast to the gepants, the CGRP mAbs are macromolecules (molecular weight ~150 kDa), as such they are unlikely to readily cross the BBB to achieve meaningful therapeutic concentrations under physiological conditions. In line with this, preclinical studies have revealed that anti-CGRP mAbs are: (i) ineffective in preventing GTN-induced allodynia when administered systemically but not intracerebroventricularly, as olcegepant did [52]; (ii) unable to penetrate the BBB in the perfused middle cerebral artery [53]; and (iii) capable of decreasing migraine-like pain with a rapid onset of action [54], which suggests that the mAbs act mostly, if not exclusively, outside the BBB.

Two recent studies with radiolabeled galcanezumab have extended our knowledge about the distribution of anti-CGRP mAbs in peripheral and central tissues of rats with uncompromised (intact) BBB. First, Johnson et al. [55] found high levels of this mAb in both the dura mater and trigeminal ganglion (rank of order of tissue level was 11 and 5% of plasma, respectively), whereas all of the central nervous system tissue levels, including CSF, were very low (<0.04% of plasma). Similarly, the study of Noseda et al. [56] found the distribution of the mAb in the dura mater, dura vasculature, and trigeminal and autonomic ganglia, but not in the central nervous system.

Even though a loss of BBB integrity could enhance the delivery of mAbs to the central nervous system as previously reported [56, 57], a recent study showed that fremanezumab was unable to prevent the induction, occurrence, or propagation of cortical spreading depression in animals where the BBB was experimentally compromised [58]. Importantly, magnetic resonance imaging studies have concluded that there is no BBB disruption during migraine attacks [59, 60]. Thus, taken together, these findings suggest that the pharmacological site of action of the mAbs to prevent the headache phase of migraine involves exclusively blockade of trigeminal nociceptive transmission at peripheral level, as the concentration of anti-CGRP mAbs that crosses the BBB is rather very limited.

As discussed above, the distribution of radiolabeled galcanezumab within the peripheral trigeminovascular system revealed specifically that the highest levels of this mAb are found throughout the dura mater (including the transverse sinus, middle meningeal artery walls, and axonal fibers), followed by the trigeminal ganglion [55, 56]. This is congruent with the trajectory of perivascular CGRP-immunoreactive sensory fibers that originate in the trigeminal ganglion, and which upon either mechanical, electrical, or chemical stimulation or during a spontaneous migraine attack release CGRP, leading to cranial vasodilation, dysfunctional nociceptive transmission, and eventually headache [1, 2, 61]. Furthermore, immunofluorescence

studies of these fibers have revealed that CGRP is mainly localized in C-fibers, whereas the components of the CGRP receptor (RAMP1 and CLR, see Fig. 2.1) were predominantly found in A δ-fibers; with expression ranging from the trigeminal ganglion, along the fiber and to the axon terminals at perivascular levels [8, 20]. Therefore, as discussed in Chap. 1, when CGRP dense-core vesicles are released from C-fibers, mAbs targeting CGRP prevent the activation of adjacent Aδ meningeal nociceptors, as proved with fremanezumab [62]. This mechanism of action would also be expected for mAbs and gepants, although through direct blockage of the CGRP receptor, but no experimental data have corroborated this yet. It is worth considering that anti-CGRP drugs might have additional (non-sensory) antinociceptive sites of action, as autonomic nerves, satellite glia, resident immune cells, fibroblasts, and dural vessels are capable of modulating the activity of meningeal nociceptors (reviewed in [63]). It is clear that further studies are needed to understand how these cells contribute to meningeal nociception and headache and to address whether anti-CGRP drugs are also targeting these sites.

In summary, current knowledge indicates that drugs capable of blocking the sensory CGRPergic nociceptive transmission at a peripheral level, reduce nociceptive input to central structures involved in craniofacial pain, alleviating craniofacial pain.

2.4 Conclusions

Drugs that block the trigeminovascular CGRPergic system are effective in the acute and prophylactic treatment of migraine. Although the complex cross-reactivity between CGRP and its family of peptides is not completely understood, current lines of evidence seem to finally have resolved the discussion on the (peripheral vs. central) antimigraine site of action of anti-CGRP drugs. The therapeutic effect of the current anti-CGRP mAbs is mainly peripheral, and this also appears to apply for gepants. Even though gepants could reach the central nervous system, different studies revealed that this site does not appear to play a prominent role in the antimigraine effects of these drugs, revealing that migraine attacks can be treated exclusively via peripheral blockage of CGRP.

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Chapter 3
Monoclonal Antibody Biology

Hsiangkuo Yuan and Stephen D. Silberstein

3.1 Introduction

Therapeutic monoclonal antibodies (mAbs) are of major importance in treating malignancy, autoimmune diseases, migraine, multiple sclerosis, and other disorders. The first therapeutic antibody (muromonab-CD3) was approved by the Food and Drug Administration (FDA) in 1986. Since then, tremendous progress has been made in antibody development allowing for greater precision in antibody pharmacology and manufacturing. Antibody-based therapeutics is one of the fastest growing areas in drug development. By 2019, there were more than 80 therapeutic mAbs available. Growing numbers of mAbs are humanized or fully human, and most are indicated for cancer or immune-related conditions (Fig. 3.1). The global therapeutic mAb market was $135.38 billion in 2018 and is expected to grow to $212.64 billion at a compound annual growth rate of 12.0% through 2022 [1]. Among the top ten drugs by global sales, seven were mAbs; adalimumab was top in sales at just under $20 billion [2].

The use of antibodies as treatment emerged in the late nineteenth century when therapeutic serum (i.e., antitoxin) was used to treat diphtheria at the Institute of Infectious Diseases (now Robert Koch Institute) by von Behring and Kitasato [3].

Disclosure: Dr. Yuan received honoraria from Supernus Pharmaceuticals, Inc.; and Trillen Medical Inc. Dr. Silberstein receives, or has received, honoraria from AbbVie.; Amgen; Aeon BioPharma; Axsome Therapeutics; Biohaven Pharmaceuticals; Cefaly; Curelator, Inc.; Epalex; GlaxoSmithKline Consumer Health Holdings, LLC.; electroCore Medical, LLC; Impel NeuroPharma, Inc.; Lilly USA, LLC; Medscape, LLC; Lundbeck; Nocera; Novartis, Inc.; Revance: Salvia, Bioelectronics; Satsuma Pharmaceuticals; Supernus Pharmaceuticals, Inc.; Teva Pharmaceuticals; Theranica; Thermaquil; and Trillen Medical Inc. No funding was received for this manuscript.

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_3

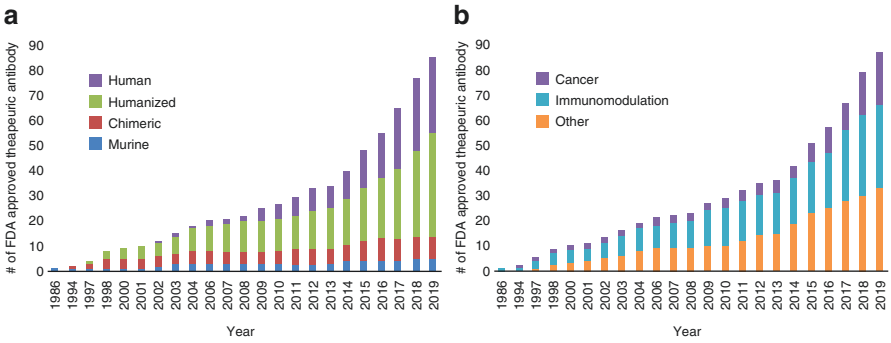

Fig. 3.1 FDA approved therapeutic monoclonal antibodies since 1986 grouped by (**a**) degree of humanization and (**b**) target class

Ehrlich postulated that the interaction between toxins and cell side-chain receptors would induce living cells to proliferate and release side chains that specifically target toxins (he coined the term "magic bullets") [4]. Since then, remarkable discoveries have resulted in 11 Nobel prizes awarded to those studying the immune system.

We now know that antibodies, also known as immunoglobulins (Igs), are large glycoproteins (~150 kDa). They can be secreted from plasma cells (free) or bound to the B cell surface (as B-cell receptor; BCR) and are responsible for humoral immunity. In a typical humoral immune response, Igs are produced by activated B cells upon interacting with either T cell-independent antigens (e.g., lipopolysaccharide, unmethylated DNA) or T cell-dependent antigens (e.g., free or bound glycoproteins). The former utilizes BCRs or toll-like receptors, and the latter requires interactions with helper T (T_H2) cells (Fig. 3.2). B cells are activated in secondary lymphoid organs involving complement activation, cytokine production, B cell differentiation, memory formation, isotype switching, affinity maturation, and plasma cell proliferation that lead to specific antibodies release, as well as opsonization and neutralization of antigens.

3.2 Structural Features of Antibodies

Five antibody isotypes exist in humans (Fig. 3.3), namely α (IgA), δ (IgD), ε (IgE), γ (IgG), and μ (IgM) with IgG being the most abundant (~75%). IgA, a dimer, is often found in mucosal area, saliva, or breast milk. IgD, a monomer, is often co-expressed with IgM on the immature B cells; it may play a role in respiratory immune function. IgE, a monomer, mediates allergic reaction and protects against parasitic infections. IgG, a monomer, can cross the placenta and is the predominant Ig in humoral immunity. IgM, a pentamer with high avidity, is the main Ig in the early phase of humoral immunity. All present as monomers on the surface of B cells functioning as BCRs. All human antibodies consist of two heavy chains (HCs) and

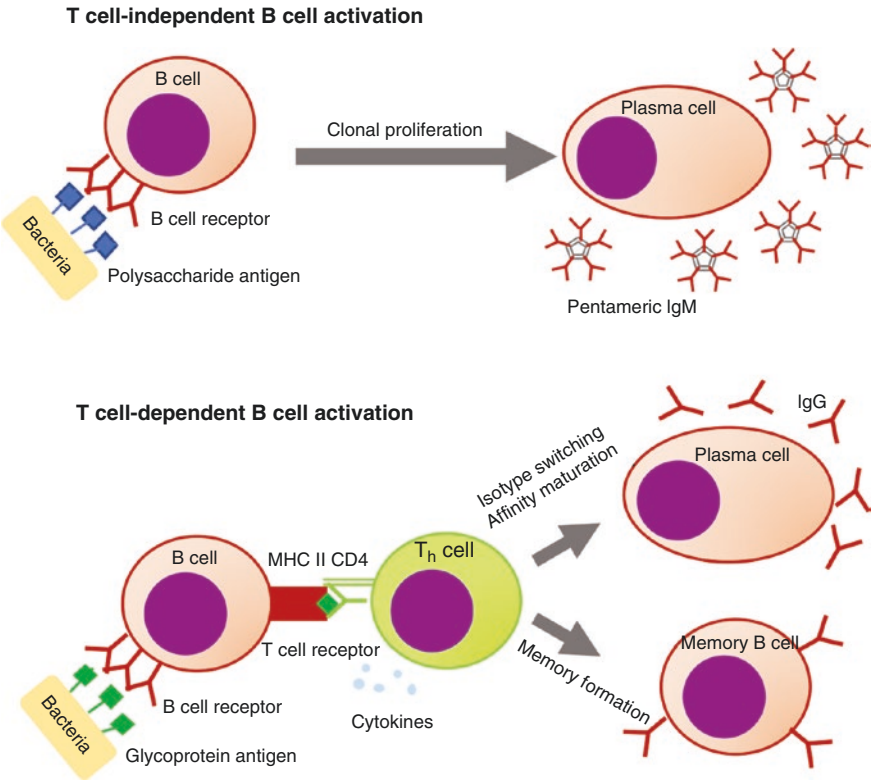

Fig. 3.2 B cell activation via T cell-independent and T cell-dependent pathways. T cell-independent response produces plasma cells secreting IgMs that share the same antigen specificity as the BCRs. In T cell-dependent pathway, naive B cells or antigen-presenting cells internalize antigens, which are presented with MHC II to T_H2 cells specific to the same antigen. Activated T_H2 cells secrete cytokines to induce B cells to proliferate and differentiate into memory B cells and plasma cells. The initial process secretes IgMs that peak around 14 days, followed by IgGs secretion. In secondary response, antibody affinity matures through somatic hypermutation and clonal selection

two light chains (LCs). Some species (e.g., camelids. Dromedaries, camels, llamas, and alpacas) and cartilaginous fishes (e.g., sharks) only have HCs. Due to the long-circulating half-life, most therapeutic antibodies and related technical discoveries are focused on IgG.

Therapeutic mAb consists of two HCs (~50 KDa) and two LCs (~25 kDa) linked by disulfide bonds into a Y shape (Fig. 3.4). Each HC contains an N-terminal variable domain (V_H) and three constant domains (C_H1, C_H2, C_H3). Between C_H1 and C_H2, there is a hinge region formed by amino acid bridges and disulfide bonds (between thiol groups from two cysteines). The LC contains a variable domain (V_L) and a constant domain (C_L). IgG can be cleaved by the enzyme papain into two antigen-binding fragments (Fabs; V_L, C_L, V_H, C_H1) and one fragment crystallizable

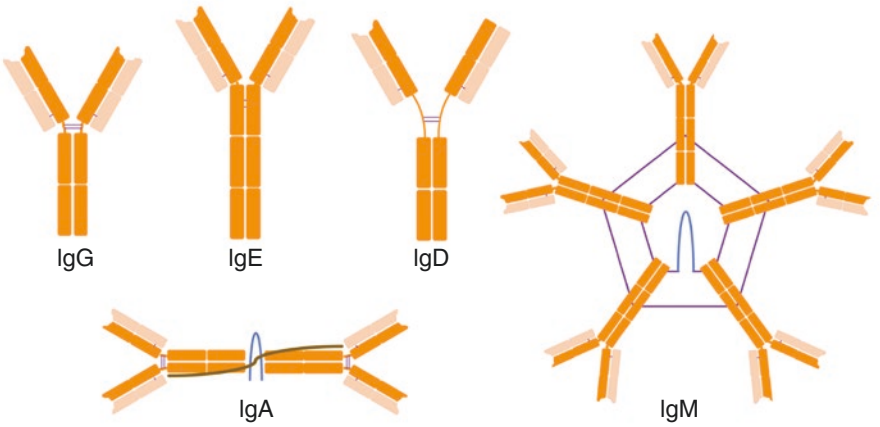

Fig. 3.3 Different types of antibodies in human. Each Ig consists of heavy chains (dark orange) and light chains (light orange) connected by disulfide bonds (purple). Compared to IgG, IgE has more heavy chains, whereas IgD has longer hinge region. J chain (blue) regulates the formation of IgA dimer and IgM pentamer. IgA is covered by a secretory component (brown) to facilitate IgA transport across the epithelium. Sizes are not to scale

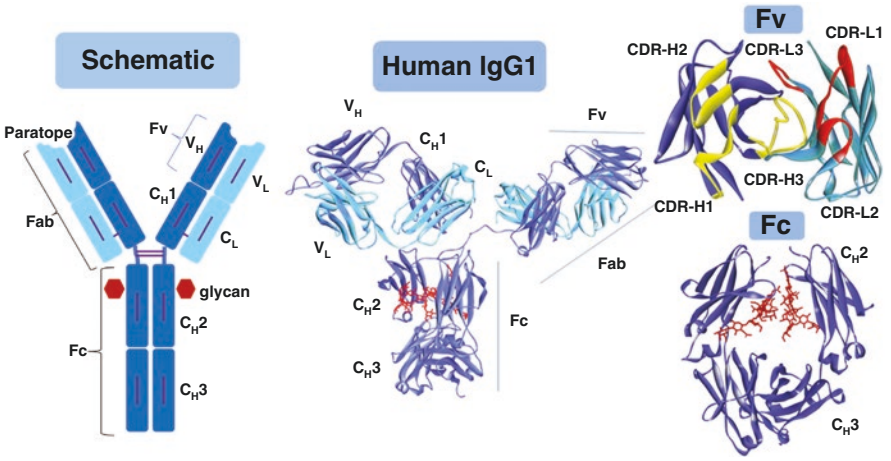

Fig. 3.4 Structure of human IgG. HC (V_H, C_H1, C_H2, C_H3) and LC (V_L, C_L) are connected by interchain disulfide bonds (purple lines). Each chain is stabilized by an intra-chain disulfide bond. The variable region (F_V) consists of six CDRs (three in V_L and three in V_H) alternate with framework regions (four in V_L and four in V_H) forming the antibody binding site (paratope) at the tip of the Fab. Two Fabs are linked to one Fc via a hinge (amino acid bridge and disulfide bonds) forming a Y-shape. The N-glycan at position 297 (red) together with C_H2 determines FcγRs binding and effector activation. Adapted from ref. [5]

region (Fc; C_H2, C_H3). Fabs contain the antigen-binding site (the paratope) in the variable region (Fv; V_H, V_L), while the Fc interacts with the surface Fc gamma receptors (FcγRs) and the complement system. Paratope binds non-covalently to the antigen site (epitope), typically a small region of peptides, lipids, or polysaccharides on the surface of antigens or antigen-presenting cells. Each paratope contains a set of six complementarity-determining regions (CDRs) that regulates antibody specificity and framework regions that support the binding of CDRs to epitope and maintain antibody structural stability. Through somatic hypermutation and the recombination of V (variable), D (diversity; only in HC), and J (joining) gene segments, diverse antibody repertoire (limited but adequate) is generated to combat foreign antigens. It has been proposed that a naive repertoire of about 10^7 different antibody structural specificities is sufficient to recognize all possible antigens [6].

In contrast, the Fc region (mainly the C_H2 domain most proximal to the hinge region) interacts with complement (e.g., C1q) and FcγRs found on the surface of many immune cells (e.g., phagocytes, natural killer cells, activated Th cells). Fc plays a significant role in effector activation, including cytokine release, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity (CDC).

Posttranslational IgG glycosylation consists of N-acetylglucosamine and mannose residues at position N297 of the HC. The heptasaccharide core at N297 can be further extended with galactose, sialic acid, fucose, and bisecting N-acetylglucosamine. N297 glycan influences the quaternary structure, stabilizes Fc, and alters the affinity of Fc to different FcγRs. The interface between the C_H2–C_H3 domains binds to the neonatal Fc receptor (FcRn), which is responsible for antibody recycling, placental passage, and antibody transport to mucosal surfaces [7]. In summary, Fv regulates target binding affinity, Fc interacts with effector via FcγR, N297 glycosylation influences immunogenicity and effector activation, and FcRn binding determines circulation half-life.

3.3 Therapeutic Antibody Types

Different types of therapeutic antibodies are available for clinical and research use. Full mAbs are the most common. Other formats include Fab, single-chain variable fragment (scFv), nanobody, bispecific antibody, antibody–drug conjugate (ADC), heavy-chain antibody, antibody–radionuclide conjugate, chimeric antigen receptor T cell, immunotoxin, and immunostimulatory antibody (Fig. 3.5). In general, due to the lack of Fc region, antibody fragments are smaller in size (in theory deeper tissue penetration) and less immunogenic than full antibodies but with reduced target binding, and increased rate of aggregation and systemic clearance. A molecule being conjugated to an antibody may alter the structural properties rendering a different pharmacology than the original antibody. In this chapter, we focus on the discussion of full mAbs.

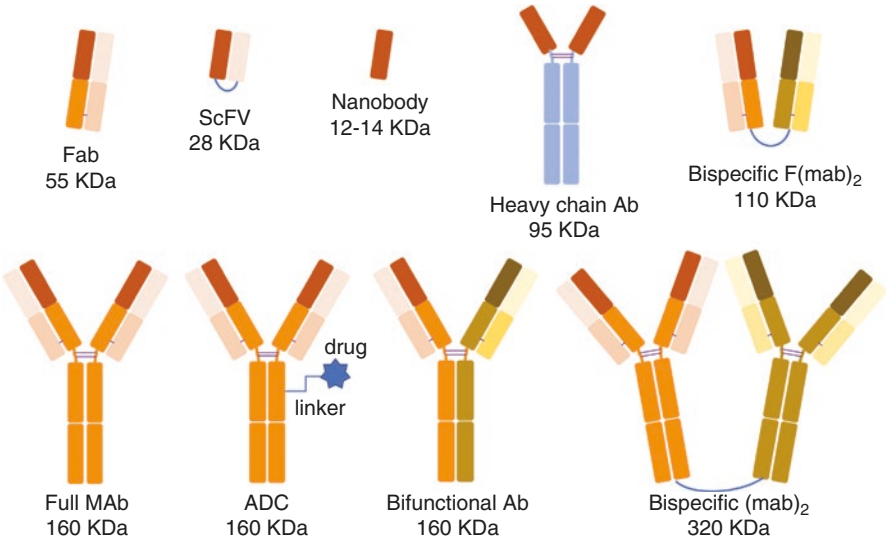

Fig. 3.5 Different types of therapeutic antibodies available for clinical use or under development

Most therapeutic mAbs are IgG, which has four subclasses named in order of decreasing abundance (IgG_1, IgG_2, IgG_3, and IgG_4). Isoelastic variants from genetic polymorphism also exist with undefined functional significance. IgGs are structurally conserved (90% homology in amino acid sequence) but differ primarily in their hinge region and N-terminal C_H2 domain leading to distinctive binding profiles of FcγR and C1q [8]. Table 3.1 summarizes the major differences between IgG subclasses. Each IgG subclass has several unique features; each bind to different FcγRs with different affinities. *IgG_1*, the most abundant subclass, is induced in response to protein antigens. It has the highest degree of hinge flexibility with one third being a T-shape conformation that shields its aggregation-prone motif and improves its physical stability [9–11]. *IgG_2* responds more to bacterial capsular polysaccharides. It has three allotypes (IgG_{2A}, $IgG_{2A/B}$, and IgG_{2B}) differing in disulfide bond distribution. IgG_{2A} is the major form in λ LC and IgG_{2B} is the major form in κ LC. High free cysteines (sulfhydryl groups) in the antibody most likely cause increased IgG_2 aggregation [12]. *IgG_3* has several allotypes. It appears in the early stage of infection, but its level does not always increase later. IgG_3 has a long hinge disulfide bonds leading to aggregation. Histidine replacement of arginine at 435 amino acid position reduces IgG_3 binding to FcRn, lowering IgG_3 recycling and decreasing its serum half-life. IgG_3 elicits high ADCC and CDC, but its short half-life limits its clinical use. *IgG_4* is predominantly expressed under conditions of chronic antigen exposure. A significant amount of IgG_4 is functionally monovalent with two different antigen-binding sites due to unstable inter-HC disulfide bonds [13]. Two different half-molecules of IgG_4 (one HC and one LC) recombine by Fab arm exchange into bispecific antibodies of reduced stability (more often behaves as monovalent antibodies). The biological relevance of this exchange is that it generates antibodies

Table 3.1 Properties of human IgG subclasses

	IgG_1	IgG_2	IgG_3	IgG_4
General				
Molecular mass (kD)	146	146	170	146
Amino acids in hinge region	15	12	62[a]	12
Inter-heavy chain disulfide bonds	2	4[b]	11[a]	2
Mean adult serum level (g/L)	6.98	3.8	0.51	0.56
Relative abundance (%)	60	32	4	4
Half-life (days)	21	21	7/~21[a]	21
Placental transfer	++++	++	++/++++[a]	+++
Antibody response to				
Proteins	++	+/−	++	++[c]
Polysaccharides	+	+++	+/−	+/−
Allergens	+	(−)	(−)	++
Complement activation				
C1q binding	++	+	+++	−
Fc receptors				
FcγRI	+++[d]	−	++++	++
$FcγRIIa_{H131}$	+++	++	++++	++
$FcγRIIa_{R131}$	+++	+	++++	++
FcγRIIb/c	+	−	++	+
$FcγRIIIa_{F158}$	++	−	++++	−
$FcγRIIIa_{V158}$	+++	+	++++	++
FcγRIIIb	+++	−	++++	−
FcRn (at pH <6.5)	+++	+++	++/+++[a]	+++

Table adapted from ref. [8]
[a]Depends on allotype
[b]For A/A isomer
[c]After repeated encounters with protein antigens, often allergens
[d]Multivalent binding to transfected cells

unable to form large immune complexes and has a low potential for inducing immune inflammation. They may act as "blocking antibody" competing against IgE [14]. Overall, IgG_1 and IgG_3 bind more efficiently and trigger more potent effector responses than IgG_2 and IgG_4. Traditionally, IgG_1 was selected as therapeutic mAb to elicit greater cytotoxicity (e.g., for killing cancer cells), whereas IgG_2 and IgG_4 were selected when lower immunogenicity was required. IgG_3 was rarely used due to its short half-life. With advances in protein engineering, these distinctive features can be modified. At this time, most commercial therapeutic mAbs are made from IgG_1 and some from IgG_2 and IgG_4.

MAbs, as the name suggests, are monovalent IgGs produced from a single B cell clone. In 1975, Milstein and Köhler developed a hybridoma technique: an immortalized myeloma cell line was fused with B cells from the spleen of the properly immunized (sufficient antibody formation) animal. This was initially a polyclonal process requiring the selection of the desired fusion cells that produce targeted

antibodies of monovalent specificity. Production initially was of low yield and limited to available myeloma cell lines (mouse or rat). Hybridomas also suffer from genetic diversity and contamination that may compromise the consistency of mAb [15]. Muromonab-CD3, the first approved mAb in 1986, was a mouse IgG_{2a} designed for mitigating kidney transplant rejection. Its use was limited due to human anti-mouse antibody response and poor pharmacokinetics [16]. Research has focused on improving binding affinity and clone consistency, reducing foreign antibodies' immunogenicity and clearance, and enhancing large-scale manufacturing. Significant breakthroughs include but are not limited to DNA- and mRNA-based immunization, biopanning, next-generation sequencing, proteomics, recombination gene editing, display libraries, transgenic animals, cell line engineering, glycoengineering, multi-parametric noninvasive live-cell analysis, high-density cell culture and harvesting, bioreactor optimization and validation, and scaled-up purification and sterilization. These technological advancements have greatly enhanced antibody production workflow, quality, consistency, and capacity. The detail of these technologies is beyond the scope of this chapter.

3.4 Monoclonal Antibody Nomenclature

The World Health Organization (WHO) established the International Nonproprietary Name (INN) system in 1950 to provide a unique (generic) name for each pharmaceutical substance. To date, except for the first mAb Muromonab-CD3, every mAb name is composed of random/fantasy prefix, substem A, substem B, and suffix. The "-mab" stem was introduced in 1990 to indicate mAb. Substems describe the disease target class (substem A) and species origin (substem B). For example, in abciximab the prefix (-ab-) serves as a unique identifier. The substem A (-ci-) denotes its cardiovascular target, and the substem B (-xi-) shows its chimeric origin. The suffix is the same -mab for every mAb. Since then, the mAb nomenclature system has been revised several times (Table 3.2, Fig. 3.6) [17–19].

The naming scheme for the substem A evolved with the technological advancement in the past two decades. The tumor subclasses were no longer divided by the target organ, and -t(u)- was replaced by -ta- to avoid confusion of "u" as human origin. New target classes (e.g., serum amyloid protein) were introduced. Longer labels were implemented for improved clarity. However, some mAbs can be used for new targets different from the original one. Single-target classification is not sufficient to describe their full potential. For instance, rituximab was initially classified for tumor use (-tu-) but later approved by the FDA for rheumatological conditions. A revision in the substem A is underway.

The initial intention for substem B was to provide information on immunogenicity based on the amount of foreign content. In the early definition, in addition to animal designations to rat (-a-), hamster (-e-), primate (-i-), and mouse (-o-), two unique designations (-xi-, -zu-) were introduced to describe the engineering methods that make the antibody more human (-u-) hence less immunogenic (Fig. 3.7). A

Table 3.2 Evolution of mAb nomenclature scheme

Substem A: target class	1997	2014	2017
Bacterial	*-ba(c)-*	*-b(a)-*	*-ba-*
Fungal		*-f(u)-*	*-fung-*
Viral	*-vi(r)-*	*-v(i)-*	*-vi-*
Infectious lesions	*-le(s)-*		
Cardiovascular	*-ci(r)-*	*-c(i)-*	*-ci-*
Serum amyloid protein			*-ami-*
Immunomodulator	*-li(m)-*	*-l(i)-*	*-li-*
Interleukin		*-k(i)-*	*-ki-*
Skeletal muscle mass related growth factors and receptors		*-gr(o)-*	*-gros-*
Neural		*-n(e)-*	*-ne-*
Bone		*-s(o)-*	*-os-*
Toxin		*-tox(a)-*	*-toxa-*
Veterinary use (pre-stem)			*-vet-*
Tumors		*-t(u)-*	*-ta-*
Colon	*-co(l)-*		
Testis	*-go(t)-*		
Ovary	*-go(v)-*		
Mammary	*-ma(r)-*		
Melanoma	*-me(l)-*		
Prostate	*-pr(o)-*		
Miscellaneous	*-tu(m)-*		
Substem B: species origin			
Rat	*-a-*	*-a-*	
Rat–mouse		*-axo-*	
Hamster	*-e-*	*-e-*	
Primate	*-i-*	*-i-*	
Mouse	*-o-*	*-o-*	
Human	*-u-*	*-u-*	
Chimeric	*-xi-*	*-xi-*	
Chimeric-humanized		*-xizu-*	
Humanized	*-zu-*	*-zu-*	

chimeric antibody (-ximab) contains foreign amino acids in the entire variable region linked to constant regions of human origin; a humanized antibody (-zumab) contains foreign amino acids in the CDRs inserted into human-derived variable regions and constant regions. With the development in antibody genetic engineering, these definitions were changed. They were based on the sequence identities of their V-gene segments available in the international ImMunoGeneTics information system (IMGT®) database (<85% human for -ximab, ≥85% human for -zumab or -umab). However, as the number of species of origin increased (e.g., Chinese hamster ovary cell, Pichia pastoris, transgenic mouse, phage display) and numerous engineering options exist beyond V-gene alteration, this labeling has become

Prefix	Infix		Sufix
Pre 2017	Substem A	Substem B	
	-t(u)-	-a-	
	-l(i)-	-u-	
abici-	-n(e)-	-xi-	-mab
	-b(a)-	-zu-	
		-vet-	
2017 revision	Substem A		
	-ta-		
	-li-		
abicidi-	-ne-		-mab
	-ba-		
	-vet-		

Fig. 3.6 The naming scheme for antibody INN prior to 2017 and the 2017 revision. In the 2017 revision, the prefix can be longer, the substem A labeling was modified, the substem B was removed, and the suffix remains unchanged

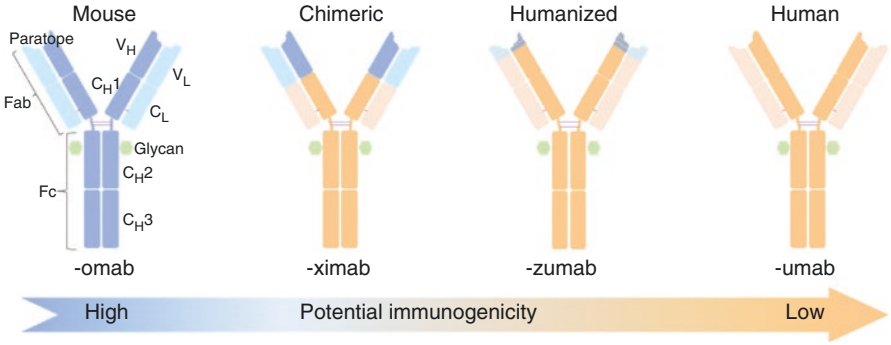

Fig. 3.7 Naming of therapeutic mAbs and their potential immunogenicity based on the origin of species

arbitrary and impractical in classifying human or nonhuman origins [20, 21]. Estimating immunogenicity based purely on the ratio of foreign content is oversimplified as many factors have roles in antibody immunogenicity. Due to (1) the increasing difficulty in identifying new, distinct, pronounceable-by-all, and not too long INN, and (2) the substem B being exploited as a marketing tool to imply advantageous immunogenicity profile even without scientific support. The INN Expert Group in 2017 recommended discontinuing the substem B except the pre-substem -vet- (used in substem A) for veterinary use [19]. For example, tafasitamab and belantamab are two recently approved mAbs using the new naming scheme.

With the development of biosimilar and interchangeable products, the World Health Assembly in 2014 adopted a resolution that mandates both Member States and the WHO Biosimilar Secretariat to facilitate access to biotherapeutic products to ensure their quality, safety, and efficacy [22]. In 2017, to separate originator

products from biosimilar products, the FDA recommended adding a distinguishing suffix that is devoid of meaning and composed of four lowercase letters to be attached with a hyphen to the core name [23]. For example, infliximab shares the core name but different suffix with other biosimilars (infliximab-dyyb, infliximab-axxq, infliximab-qbtx, infliximab-abda). At the time of writing, the FDA has approved 28 biosimilar products and 19 of them are mAbs [24].

3.5 Pharmacodynamics (PD) and Pharmacokinetics (PK)

MAbs, a macromolecule, behave differently than small molecules (Table 3.3). MAbs have low nanomolar or picomolar target affinity (they bind tightly to their target), which is several fold higher and specific than small molecules (they bind loosely to their target). Their main pharmacodynamic (PD) actions are: (1) binding to soluble ligand interfering with signal transduction or toxicity, (2) binding to surface protein/receptors preventing their functions, hampering receptor dimerization, or causing receptor downregulation, and (3) indirect effector activation-inducing ADCC, ADCP, and CDC. Target binding is a dynamic process governed by the laws of thermodynamics favoring association over dissociation. It does not require an exact lock-and-key fit as protein conformational plasticity allows for induced fit [25]. In an equilibrium PK/PD model, stronger affinity, less dissociation, higher dose, and slower clearance lead to a greater and longer suppression of free ligand concentration [26]. As a ligand binder, mAbs likely act as a damper (may not affect baseline function but dampens the reactive surge). As a receptor binder, mAbs may not always bind in an "one-to-one" fashion. Antibody bivalence, stoichiometry, lipid raft, FcR interaction, and local pH, all play a role in receptor binding [27].

Table 3.3 Monoclonal antibody versus small molecule

	Monoclonal antibody	Small molecule
Size	~150 kDa	<1 kDa
Target specificity	High	Low
Target location	Mostly extracellular	Intracellular/extracellular
Off-target effect	Low	High
Drug interaction	Low	High
Distribution mechanism	Extravasation	Extravasation, diffusion, lipophilic transport
Membrane permeability	Low	High
Preferred administration	Parenteral	Enteral, parenteral
Half-life	Days to weeks	Hours to days
Clearance sites	Vascular endothelial cells, reticuloendothelial cells	Liver, kidney
Metabolism system	Peptidase, proteinase, hydrolase	Oxidases, conjugating enzymes
Immunogenicity	High	Low

Since mAbs typically have little off-target binding, both effect and adverse effect come from its action on the target. For example, B cells suppression can be used for lymphoma treatment but can cause systemic immunosuppression; antiangiogenic antibodies can cause hematological or vascular toxicities.

3.5.1 Absorption

MAb, a macromolecule made of amino acids, requires parenteral access via intravenous (IV) or subcutaneous (SC) but not enteral administration (to avoid gastrointestinal degradation, an exception is newborns). IV injection allows greater bioavailability, SC injection is more convenient. MAbs given SC need to be absorbed via the lymphatic system (not the capillaries) and are discharged by the right lymphatic and thoracic ducts into the venous system. Factors such as size and positive surface charge, as well as local proteolysis and immunophagocytosis, can lead to tissue degradation and delay mAb absorption into the circulation. The T_{MAX} (time to reach maximal serum concentration [C_{MAX}]) occurs at the end of IV infusion with 100% bioavailability. In contrast, it takes 3–8 days after SC injection to reach C_{MAX} with 40–80% bioavailability. Permeation enhancers, such as recombinant human hyaluronidase, have been used to improve bioavailability [28].

3.5.2 Distribution

MAbs have different distribution kinetics than small molecules. Distribution is governed by active and passive transport across multiple biological barriers. While some receptors facilitate the uptake of mAbs (active mechanism), most mAbs are transported passively, influenced by factors related to mAb (hydrodynamic size, polarity, lipophilicity, charge), barrier (permeability, thickness, surface area), and circulation (blood flow characteristics, concentration/pressure gradient). Since all mAbs are IgGs of similar structure (large, lipophobic), their distribution depends primarily on the hydrostatic/oncotic pressure gradient and the extravasation (passive convection) via fenestrated capillary or sinusoidal cleft. MAbs are largely confined in the bloodstream and distributed in organs with leaky vasculature such as tumor, thyroid, skin, liver, and spleen. Non-leaky vasculature limits mAb passive transport. In the brain parenchyma, due to the tight blood–brain barrier (BBB), mAb concentration is very low (0.2%) relative to plasma concentration. However, in the central nervous system, the dura mater, pituitary, and circumventricular organs are much leakier than regions inside the BBB or brain–CSF barrier (arachnoid mater). Sensory ganglia (trigeminal, cervical, vagus, sphenopalatine, etc.) are highly permeable but with interganglionic differences [29]. Study has shown that injected mAbs accumulate to a greater extent in dura mater (11%) and trigeminal ganglion (5.2%) than in cortex (0.23%), hypothalamus (0.34%), or cerebrospinal fluid (0.12%) [30]. Inside the ganglia, mAbs are found surrounding individual neurons possibly on satellite glial cells [31].

3.5.3 *Elimination*

MAbs, in contrast to small molecules, are neither filtered by the kidney nor metabolized by microsomal/mitochondrial oxidation in the liver. Rather, they are metabolized inside vascular endothelial cells and by the reticuloendothelial system (e.g., Kupffer cells, monocytes, macrophages) throughout the body. Human IgG catabolism is estimated to be 33, 24, 16, and 12% respectively from skin, muscle, liver, and gut [32]. This may explain why body surface area or body weight is often used to guide mAb dosing. The decision for fixed-dosing or body size-based dosing varies by mAb [33]. MAb is transported intracellularly by receptor-mediated endocytosis (minor) or pinocytosis (major). Receptor-mediated endocytosis occurs at the target cell (target-mediated drug disposition [TMDD]) or immune cell surface; pinocytosis utilizes a nonspecific fluid-phase endocytosis of the mAb into endosomes. By TMDD, mAb clearance may be nonlinear depending on dose level, internalization rate, target density, and turnover [34]. In contrast, nonspecific pinocytosis is usually dose-independent since most therapeutic antibodies concentrations fall below endogenous IgG concentration (~10 g/mL). The contribution of FcγR binding to mAb elimination remains to be studied.

Once in intracellular endosomes, mAbs can: (1) undergo enzymatic degradation (inside endosomes) into small peptides or amino acids, or (2) recycle back to circulation via a salvage pathway mediated by neonatal Fc receptor (FcRn) (Fig. 3.8). FcRn, a nonclassical MHC 1 receptor, is highly expressed in skin, muscle, liver, and spleen, where vascular endothelial cells and reticuloendothelial cells are the major

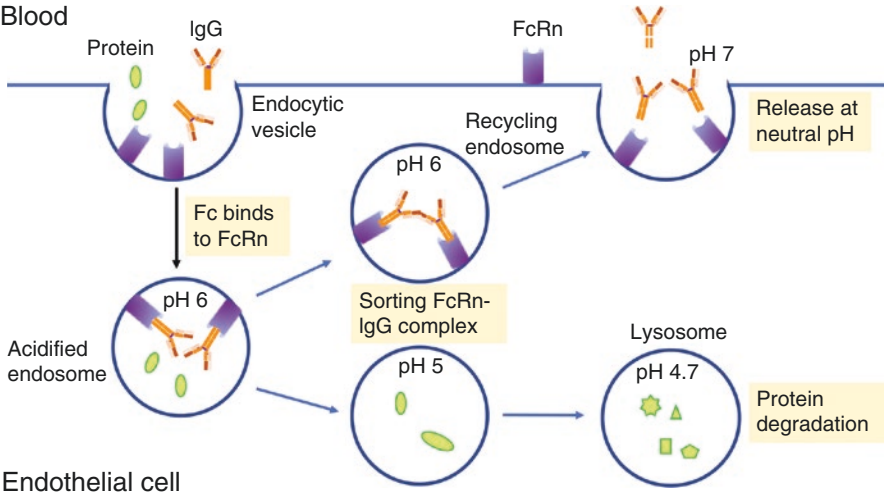

Fig. 3.8 FcRn-mediated pH-dependent IgG salvage pathway. FcRns are expressed on vascular endothelial cells and circulating reticuloendothelial cells (e.g., monocytes). Once internalized by pinocytosis (fluid-phase endocytosis), FcRns bind to IgGs in an acidic environment, protects IgGs from entering lysosome and releases IgGs under neutral pH. This pathway recycles endocytosed IgGs back to circulation and extends IgGs' serum half-life

sites of IgG metabolism [7]. This recycling process is the main factor responsible for the extended IgG circulating half-life (approximately 3 weeks). Without FcRn, IgG catabolism is similar to that of IgM or IgA (5–6 days) [35]. Fab and scFv due to the lack of proper binding to FcRn have a much shorter half-life (0.5–30 h). In fetuses and neonates, FcRn mediates IgG transport via the placenta and intestine, respectively. FcRn is expressed in the brain microvascular endothelium and choroid plexus epithelium; it facilitates the transport of IgG from the brain parenchyma and cerebrospinal fluid, respectively, back to blood vessels [36]. This salvage pathway is saturable upon large-dose pooled IgG administration but probably does not affect steady-state antibody clearance under a typical therapeutic mAb dose regimen [37]. Higher doses of IgG are cleared faster due to aggregation. In certain situations, increase in half-life can be seen in mAbs targeting internalizing membrane antigens with high tissue expression. Such an antigen-dependent clearance pathway is called "antigen sink." In short, mAbs are metabolized intracellularly but can be salvaged by FcRn to extend their circulation half-life.

For most mAbs, no populational clearance difference was observed by age, sex, renal function, or hepatic function. At an individual level, significant variation exists in PK between individuals with the same mAbs or even the same individual over time [38]. Although there is no direct drug–antibody interaction, certain immunomodulating drugs (methotrexate, azathioprine, mycophenolate) may affect FcγR expression, thereby affecting mAbs' metabolism. Statin and fibrate also induce the expression of proprotein convertase subtilisin/kexin type 9 (PCSK9), increasing the clearance of anti-PCSK9 antibodies [39]. Overall, mAb's unique PK allows for a longer dosing interval with no adjustment need for hepatic/renal dysfunction, but each individual's response may vary.

3.6 Safety and Immunogenicity

MAbs can have safety issues, including immunosuppression (e.g., leukopenia), infections (e.g., reactive tuberculosis, progressive multifocal leukoencephalopathy), autoimmune diseases, thrombotic diseases, malignancies, dermatitis, and even cardiotoxicity [40]. In a post-marketing analysis of all novel therapeutics from 2001 to 2010, biologics were associated with greater frequency of safety events (incidence rate ratio: 1.93, 95%CI 1.06–3.52) [41]. Many of these adverse effects were related to their mechanism of actions (so-called on-target risks) that are commonly associated with anticancer or immunomodulating mAbs [42, 43]. However, immune reactions (e.g., injection site reaction, acute infusion reaction, hypersensitivity reaction, serum sickness, cytokine release syndrome) toward exogenous protein are not uncommon. Immunogenicity is another issue for mAbs.

The formation of the immune response to mAb depends on multiple factors, including mAb's structure (nonhuman component, glycosylation, impurity, aggregation, deamidation), administration (route, frequency, dose, storage, excipient), and patient's condition (genetic, underlying diseases, concomitant medication) [44]. Immunogenicity to the chimeric/humanized part or idiotopes on human

antibody can generate antidrug antibody (ADA) that has an impact on mAb's safety. ADAs can produce administration reactions, alter mAb PK, affect target binding, reduce efficacy, and very rarely cause anaphylactic reactions. ADA can be generated by either a T-cell dependent or independent pathway. In T-cell independent pathway, mAb aggregates and impurities may increase the number of adjacent epitopes crosslinking to BCRs. In T-cell dependent pathway, mAb may be internalized by antigen-presenting cells then presented to T cells to activate plasma cell proliferation. ADAs can be neutralizing or non-neutralizing. Neutralizing ADAs reduces mAb's specific binding to their target affecting efficacy; non-neutralizing ADAs forms ADA–mAb immune complex that can trigger various downstream effects in addition to being cleared by the reticuloendothelial system [45]. The presence of ADAs may affect mAb concentration and even clinical efficacy. More studies are needed to understand ADA generation mechanism so as to design methods to lower immunogenicity and improve mAb safety [46].

3.7 Conclusion

MAbs are of tremendous value in modern therapeutics with continuous growth in the number of approved mAbs and their global demand and market. They offer a substantial advantage toward small molecules by having greater target affinity, longer circulating half-life, and minimal off-target adverse effects. MAbs are particularly useful for targets in the circulation or near the extravasation sites but not yet in areas of limited vascular permeability. Critical understandings in the immune regulation as well as antibody's biology and its engineering have continued to bring new technology for refining mAbs' quality and manufacturing, as well as expanding their clinical potentials. Numerous strategies have also been exploited to identify new targets, to optimize mAb PD/PK, to enhance safety profile, and most of all, to develop the next-generation biotherapeutics for improved patient care.

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Chapter 4
Guidelines for Clinical Trials

Raffaele Ornello, Eleonora De Matteis, and Simona Sacco

Clinical trials are the mainstay of evidence-based medicine and ensure that research is well-conducted. The patient-reported and often unpredictable nature of headache disorders represents a challenge in designing randomized controlled trials (RCTs). Nevertheless, the last decade has seen the advent of important advances in evidence-based headache treatment.

In this chapter, we will assess and summarize the available guidelines for clinical trials in headache disorders with special reference to anti-CGRP(r) mAbs. We will also provide suggestions for future well-designed studies.

For a more complete description of RCT guidelines, we refer the reader to the international guidelines for the prevention of migraine [1–3] and cluster headache [4]. In the present chapter, we will discuss the aspects of patient selection, operating procedures, and outcome assessment that are relevant for anti-CGRP(r) mAb treatment. Table 4.1 shows the advantages and potential drawbacks of performing RCTs of those drugs.

4.1 Patient Selection

4.1.1 Headache Definition

All the available guidelines recommend that the definitions of disease follow the International Classification of Headache Disorders (ICHD) criteria.

The third edition of the ICHD criteria [5] identifies chronic migraine as a distinct entity, thus leading to specific trials on chronic migraine. This is a relevant aspect

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_4

Table 4.1 Specific characteristics of monoclonal antibodies acting on the calcitonin gene-related peptide or its receptor, their potential advantages, and drawbacks in randomized controlled trial design

	Advantages	Drawbacks
High selectivity for a migraine-specific mechanism	Clear definition of the population who potentially benefits from the drugs	Uncertain benefit for non-migraine headaches comorbid with migraine
No known interactions	Possibility of widening the inclusion criteria to patients with comorbidities or concomitant medication	Interactions might emerge in the future with long follow-up of wide cohorts
Monthly or quarterly subcutaneous administration	Excellent monitoring of compliance (noncompliance is virtually absent)	Patients might prefer oral to injectable drugs, especially if only partially effective
Early onset of efficacy	Patient satisfaction; better monitoring of efficacy endpoints	It might push towards trials with short observation periods
Low adverse event rate	Patient satisfaction; potentially high enrolment rates	Prolonged follow-up times and huge cohorts are required to better identify adverse events

for anti-CGRP(r) mAbs as they are effective in both episodic and chronic migraine. A relevant issue for chronic migraine is the association with medication overuse. The available guidelines state that the inclusion of patients with medication overuse in RCTs can be safe, provided that patients do not overuse barbiturates or opioids, which they do not have medical complications due to overuse such as peptic ulcer from nonsteroidal analgesic overuse, and that adequately powered subgroup analyses are performed on such patients [2].

As mAbs act on the CGRP pathway, they could exert an effect on some trigeminal autonomic cephalalgias and especially on cluster headache. The international guidelines for trials on cluster headache, although outdated, also recommend staying to the ICHD definitions [4]. As well as migraine, cluster headache is also classified into an episodic and a chronic form. Given the poor predictability of episodic cluster bouts and the poor efficacy reported for anti-CGRP mAbs in episodic cluster headache [6], it is likely that future RCTs with mAbs will be performed in patients with chronic cluster headache.

An interesting issue is the frequent comorbidity between migraine and other headache disorders.

Migraine is frequently associated with other CGRP-dependent headache disorders, such as cluster headache, which might also benefit from anti-CGRP(r) treatments. Comorbid headaches do not exclude patients from RCTs, provided that patients are able to distinguish the different headache types [2, 3]. Preliminary real-life evidence also shows that anti-CGRP(r) mAbs can be effective on comorbid migraine and cluster headache [7]. Tension-type headache is also frequently comorbid with chronic migraine; patients with comorbid chronic migraine and tension-type headache can be included in trials provided that they fulfill the diagnostic criteria for chronic migraine [2].

The ICHD criteria are designed for adults, but also proved reliable in children and adolescents and might be used for RCTs also in that age group [1].

4.1.2 Age and Sex

The available RCTs of anti-CGRP(r) mAbs in migraine enrolled male and female adult patients aged from 18 up to 70 years [8, 9], while the published trial of mAbs in cluster headache included patients aged 18–65 years [6]. The exclusion of patients with migraine aged over than 70 years is justified by the vascular safety concerns of inhibiting a potent vasodilator like CGRP. The results of RCTs and their open-label extensions suggest that anti-CGRP(r) mAbs have a favorable vascular risk profile [10–12]. However, data from RCTs should be interpreted with caution and be confirmed by real-life data.

As migraine is a predominantly female health concern [13], it is advisable to enroll more women than men in RCTs. Factors such as the specific vascular risk profile of women, in whom migraine is more prevalent than men, should be taken into account when assessing the safety of blocking CGRP [14].

4.1.3 Comorbidities and Concomitant Medications

As well as RCTs performed for any condition, those of anti-CGRP(r) mAbs excluded patients with major medical and psychiatric comorbidities. The issue of comorbidities is relevant in patients with chronic migraine in whom obesity, anxiety, depression, and chronic pain are common [15–18]. As a measure of precaution, patients with significant comorbidities should be excluded from RCTs of anti-CGRP(r) mAbs. However, the high specificity safety profile of anti-CGRP(r) mAbs, which were shown in the available trials and open-label extensions might pave the way for a more liberal use of those drugs. Patients whose comorbidities might negatively influence the trial results because of compliance issues or potential confounding.

The use of concomitant medications is an important issue in the management of patients with migraine. Monotherapy is the best way to assess the effect of preventive drugs. However, the concomitant use of a preventative drug might be of help in patients with chronic migraine, provided that the drug is taken at a stable dose for at least 3 months and kept unchanged for the duration of the entire trial [2]. This approach can be particularly useful in trials of anti-CGRP(r) mAbs, whose specific action does not interfere with that of other preventatives. It is important to provide stratified analyses according to concomitant preventive medication use.

4.1.4 Headache Onset and Duration

The available guidelines unanimously recommend that migraine be present for ≥12 months before enrolling subjects in trials and that age at migraine onset be <50 years [2]. Those criteria have been established to avoid the potential inclusion of patients with secondary migraine-like headaches.

4.1.5 Previous Treatments

The available guidelines state that patients with previous preventive treatment failures can be included in trials on chronic migraine [2]. The early trials on anti-CGRP(r) mAbs were performed in patients with a limited amount of preventive treatment failures in their history. However, the most recent trials, including LIBERTY on erenumab [19], FOCUS on fremanezumab [20], and CONQUER on galcanezumab [21], focused on patients with two to four preventive treatment failures in their history. The population of patients with resistant or refractory migraine, in which most or all preventive treatments have failed [22], is interesting to test the novel migraine-specific treatments.

4.2 Operating Procedures

All the available guidelines agree that trials of anti-CGRP(r) mAbs should be randomized and have a double-blind design. A parallel-group design is preferable over a crossover design, as it ensures the absence of carryover effect and allows shorter trial durations. On the other hand, a crossover trial provides higher statistical power with the same number of participants [2, 3].

4.2.1 The Issue of Comparison with Placebo

Any active treatment in migraine should provide a comparison with placebo. Placebo response is always substantial in migraine trials and might hinder the assessment of the efficacy of migraine preventive drugs. On the other hand, superiority of a migraine preventive over an active comparator does not mean superiority compared with placebo. Therefore, even RCTs comparing anti-CGRP(r) mAbs to an active treatment should use a placebo arm.

Anti-CGRP(r) mAbs are particularly suitable for RCTs due to their specific action. Any active comparator would be not specific for migraine, with an unclear mechanism of action, and possibly contraindicated in many patients. On the other

hand, mAbs could be interesting active comparators to design RCTs for future treatments. RCTs on future treatments will likely consider including patients with no response, adverse events, or contraindication to anti-CGRP(r) mAbs.

4.2.2 Screening and Baseline Phase

The available RCTs of anti-CGRP(r) mAbs were performed in patients with episodic or chronic migraine. To be classified in those categories, patients need to recall their headaches over the previous 3 months. Guidelines suggest that after the initial screening patients should be followed up prospectively for at least 28 days before initiating experimental treatments [2]. This amount of time is needed to confirm the initially declared frequency of headache and migraine. Given the monthly administration of most anti-CGRP(r) mAbs, 1 month of baseline facilitates the assessment of drug efficacy.

4.2.3 Trial Duration

Guidelines suggest that the optimal duration of the placebo-controlled phase of RCTs on migraine drugs is 12–24 weeks [2]. This suggestion is also valid for anti-CGRP(r) mAbs, even if the excellent safety profile of those drugs has shifted the interest toward the assessment of prolonged treatments. After the randomized double-blind phase, a long-term assessment of adverse events [2] is reasonable and especially for drugs associated with few adverse events such as anti-CGRP(r) mAbs.

4.2.4 Open-Label Extensions

Migraine is a chronic condition which often requires prolonged preventive treatments. The available oral preventatives for migraine are limited by poor long-term tolerability, while anti-CGRP(r) mAbs are more suitable for prolonged administration [10]. Besides, as stated above, anti-CGRP(r) mAbs have an excellent safety profile with a low incidence of adverse events; therefore, some adverse events might be overlooked during the first months of treatment and emerge in the long term. For those reasons, open-label extensions of RCTs are advisable for anti-CGRP(r) mAbs. Preliminary results of open-label extensions have already been published, largely confirming the safety and efficacy of the drugs [23, 24].

4.3 Endpoint Assessment

It is essential to assess primary and secondary endpoints by means of a headache diary, be it on paper or electronic.

4.3.1 Definitions

4.3.1.1 Migraine Day

A migraine day is defined as ≥4 h of moderate-to-severe headache fulfilling the internationally defined criteria of migraine with or without aura [5] or responding to acute migraine-specific medications such as triptans or ergotamine derivatives.

4.3.1.2 Headache Day

A headache day is defined as a day of moderate-to-severe headache not fulfilling the criteria for migraine. It is relevant to identify non-migraine headache days in patients treated with anti-CGRP(r) mAbs as some of them might in fact be the transformation of previous migraine days, although this issue is a matter of debate. RCTs usually exclude patients that cannot distinguish between migraine and any headache; however, many patients and even physicians cannot often distinguish between migraine and non-migraine headache.

4.3.1.3 Responder Rate

Responder rates are the proportion of patients with a predetermined percent reduction in migraine days or moderate-to-severe headache days during the study period [2]. The assessed percentage reduction is usually 50%; however, other percentages might be considered, including 30, 75, and 100% [2]. Anti-CGRP(r) mAbs proved to be among the most effective migraine preventatives; therefore, the assessment of ambitious endpoints such as the 75 or 100% responder rates might be justified. Another endpoint to assess might be the proportion of patients with absolute decrease in monthly headache days up to preplanned cutoffs such as 4 monthly headache days.

4.3.2 Headache-Related Endpoints

Headache-related primary endpoints include the assessment of decrease in monthly migraine days or monthly headache days or responder rates [2]. If one of those endpoints is not chosen as primary, it might be assessed as secondary.

Headache or migraine intensity is commonly assessed by using a 4-point categorical rating scale or an 11-point Visual Rating Scale [2]. Headache intensity is commonly assessed as a secondary endpoint; however, it might be relevant to patients as it has an impact on functional impairment and acute medication use. The same is valid for the assessment of total headache and/or migraine hours.

Additional headache-related endpoints that might be assessed include conversion to episodic headache in patients with chronic migraine and onset of effect during the first weeks of treatment [2]. Anti-CGRP(r) mAbs have shown a rapid onset of efficacy which is worth investigating in further RCTs.

4.3.3 Assessment Timepoints

The available RCTs of anti-CGRP(r) mAbs all had a duration of 12–24 weeks; however, the timepoints for outcome assessment were defined in different ways [8]. Assessing outcomes over the entire study period can provide an estimate of the overall efficacy of anti-CGRP(r) mAbs. However, given the potentially delayed efficacy onset of those drugs, assessing efficacy after some months from treatment start is advisable. If RCTs last 24 weeks, it is reasonable to assess the efficacy endpoints over weeks 12–24 [2]. As the results of the different RCTs should be comparable, it is advisable to conduct the analyses in the same way across trials.

4.3.4 Acute Medication Use

Acute medication use is an important outcome in RCTs of migraine preventive agents. Guidelines recommend assessing the days of use of acute migraine-specific drugs, the days of use of any acute drugs, and the number and proportion of patients withdrawing medication overuse [2]. Well-structured headache diaries should also capture the number of acute drug doses taken, to better assess headache attack duration and response to medication. A reduction in the number of acute drugs taken suggests an improvement in the patients' clinical status even if monthly migraine days or days of acute medication do not decrease.

4.3.5 *Patient-Reported Outcomes*

The assessment of patient-reported outcomes is an emerging aspect of RCTs of migraine preventive agents. Those outcomes are reported as validated questionnaires encompassing several dimensions of the patients' status, including symptoms of depression and anxiety, functional impairment, and perceived quality of life [2]. A list of instruments and their characteristics is presented in Table 4.2. All those scales were used in RCTs of anti-CGRP(r) mAbs. There are no preferred

Table 4.2 Commonly used instruments to assess patient-reported outcomes

Instrument	Dimensions assessed	Description
Patient global impression of change (PGIC) [27]	Patient satisfaction	7-point Likert scale + 11-point Visual Rating Scale
Functional Impairment Scale (FIS) [28]	Impairment during daily activities	4-point scale
Migraine Functional Impact Questionnaire (MFIQ) [29]	Physical, social, and emotional functioning	0–100 score on four domains
Patient Health Questionnaire-9 (PHQ-9) [30]	Depression	Nine-item questionnaire based on the DSM-IV diagnostic criteria; 4-point Likert scale; 2-week recall
Beck Depression Inventory (BDI) [31]	Depression	21-item inventory; 4-point Likert-type scale
State-trait Anxiety Inventory (STA-I) [32]	Anxiety	40-item inventory; 4-point Likert-type scale
Generalized Anxiety Disorder (GAD-7) [33]	Anxiety	7-item questionnaire; 4-point Likert-type scale
Patient Health Questionnaire-4 (PHQ-4) [34]	Depression/anxiety	Questionnaire in four items, two on depression, and two on anxiety; 4-point Likert-type scale; 2-week recall
Hospital Anxiety and Depression Scale (HADS) [35]	Depression/anxiety	14-item inventory (7 on anxiety, 7 on depression)
Migraine Disability Assessment Questionnaire (MIDAS) [36]	Migraine-related disability	5-item questionnaire + 2 additional items; 3-month recall
Headache Impact Test (HIT-6) [37]	Migraine-related disability	6-item questionnaire; 1-month recall; 5-point Likert-type scale; needs license
Migraine-Specific Quality of Life Questionnaire (MSQ v2.1) [38]	Migraine-specific quality of life	14 items for three domains—Role function–preventive (RP), role function–restrictive (RR), and emotional function (EF)
EuroQoL-5 Dimension Questionnaire (EQ-5D) [39]	Quality of life	5-Item questionnaire; needs registration
Short-Form 36-item Health Survey [40]	Quality of life	Non-migraine-specific tool of 36 items in eight scales

instruments in this regard; however, the instruments used in RCTs should be validated. Despite validation, however, those scales are affected by recall bias. There is a need for patient-reported outcomes of higher quality and reliability that can be useful in improving the patients' care.

4.3.6 Adverse Events

As stated above, the assessment of adverse events of anti-CGRP(r) mAbs should be extremely detailed, given the excellent safety profile of those drugs. In RCTs, the adverse events of the drugs should be carefully and frequently assessed. As the number of available RCTs of anti-CGRP(r) mAbs increases, adverse events from each of those drugs are becoming more and more predictable. Preliminary reports suggest that the use of checklists encompassing the most frequently reported adverse events might help identifying unnoticed adverse events in patients treated with anti-CGRP(r) mAbs [25].

4.4 The Issues of Cluster Headache Trials

Performing RCTs in patients with cluster headache is difficult for several reasons. First, it is a relatively rare headache; hence, it is difficult to recruit large cohorts of patients in RCTs and it is almost impossible to stratify statistical analyses by specific subgroups. Second, the most frequent subtype of cluster headache is the episodic subtype, in which short-lasting headache clusters occur over periods of weeks or months, followed by prolonged headache-free intervals. For those reasons, RCTs for the prevention of cluster headaches are usually small-sized. Besides, it is difficult to prove a preventive efficacy of cluster headache preventive treatments, unless they have a very early onset of efficacy. Anti-CGRP(r) mAbs are the ideal candidates for cluster headache prevention, as they inhibit the CGRP pathway involved in cluster headache [26] and have an early onset of action.

The available guidelines to perform RCTs in cluster headache, although outdated (1995), are still valid [4]. In the same way as those for migraine, guidelines for RCTs in cluster headache recommend including patients through a screening, a baseline, and a double-blind, randomized, placebo-controlled treatment period. Given the short duration of single cluster attacks, the efficacy assessments should rely on the weekly frequency of those attacks.

An example of the issues of RCTs in cluster headache is the trial of galcanezumab in episodic cluster headache [6]:

1. The RCT involved 35 sites in Europe and North America. An international collaboration is key to ensure the success of RCTs of anti-CGRP(r) mAbs in cluster headache.

2. Despite the high number of involved sites, the trial was halted before reaching the planned sample size because of a lower-than anticipated number of patients entering into an active cluster headache period during the screening period. The unpredictability of cluster headache challenges the feasibility of adequate RCTs. Of note, only 106 (33.8%) of the 314 screened patients were randomized.
3. Despite showing efficacy over the first month of treatment, galcanezumab failed to show a significant efficacy over the second month of treatment because of the spontaneous cessation of cluster bouts in the placebo arm. Besides, placebo response was high, with 53% of patients reporting a reduction of ≥50% in the weekly attack frequency of attacks [6].

4.5 Conclusions and Future Perspectives

For the first time in the history of migraine treatment, we assisted to the growing of a line of clinical research into migraine-specific migraine treatments: anti-CGRP(r) mAbs. The RCTs performed on those drugs have been performed according to a robust methodology. In this chapter, we summarized the current recommendations to perform RCTs. However, the changing landscape of migraine prevention might require adjustments to current practice. The new drugs may also be used to prevent cluster headache, even if the design of RCTs for that condition poses several challenges. Nevertheless, resolving those challenges might pave the way to designing robust RCTs of anti-CGRP(r) mAbs for cluster headache and for all those rare headaches in which basic research identifies the role of CGRP.

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Chapter 5
Human Models

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5.1 Introduction

Experimental human headache models provide a unique and powerful scientific advantage in the study of the complex pathophysiological mechanisms underlying headache disorders [1, 2]. While preclinical headache models provide valuable information on the pathophysiological mechanisms [3], they hold limitations as migraine is considered a uniquely human experience.

A migraine is characterized by recurrent severe headaches that can be treated by acute antimigraine drugs. Remarkably, experimentally induced migraine attacks mimic the patients' usual attacks and are treatable by antimigraine medication [1, 2]. A unique feature of these models is that only individuals with migraine develop "triggered" attacks, while healthy participants solely develop a mild headache. The current concept of experimental human headache models was validated and established in the early 1990s, where infusion of glyceryl trinitrate (GTN), a nitric oxide donor, induced attacks in migraine patients [4, 5]. Later, an array of pharmacological and non-pharmacological experimental triggers have been applied to investigate various aspects of the migraine mechanisms and to identify new potential drug targets (Fig. 5.1) [1, 2]. To this end, human models of migraine have been essential in the development of therapies targeting calcitonin gene-related peptide (CGRP) or its receptor [6, 7]. This chapter summarizes discoveries originating from human models of migraine with a focus on CGRP.

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_5

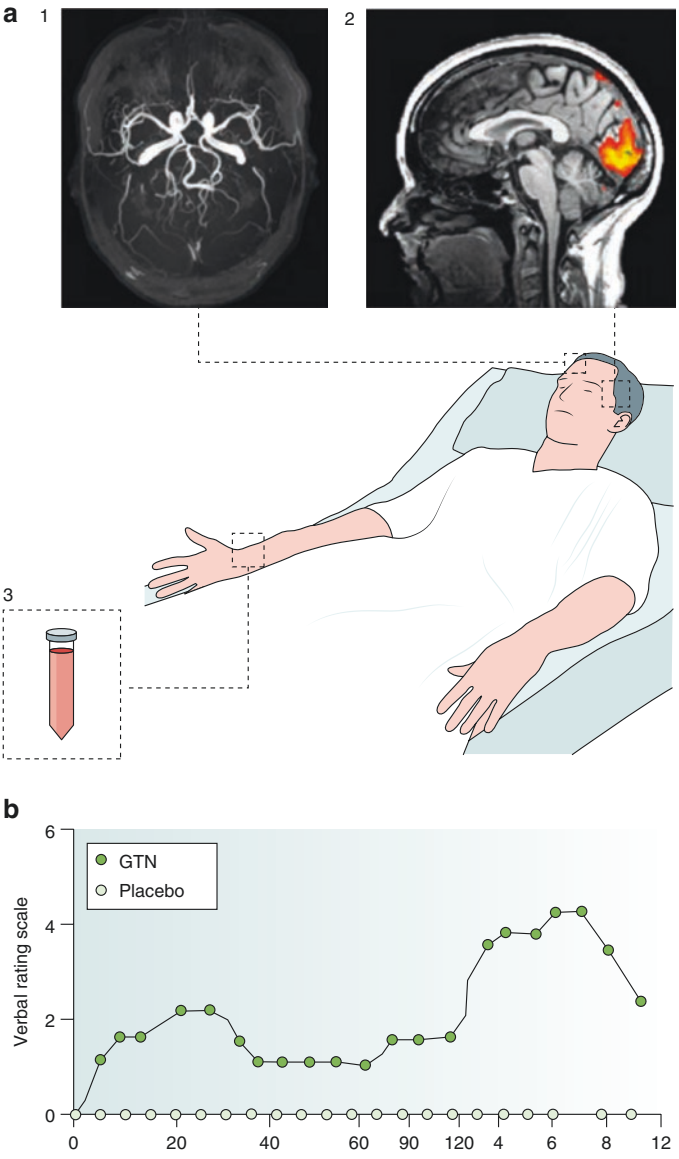

Fig. 5.1 The human model of migraine. (**a**) A range of modalities are used to detect hemodynamic effects of the infusion, which might include the intracranial and extracranial arteries [(1) magnetic resonance angiography] or brain activity [(2) blood oxygen level-dependent functional MRI (fMRI)]. These may be recorded at baseline and predefined intervals after intervention. Vital signs, such as heart rate and blood pressure, are measured continuously throughout the study. Studies can be tailored to assess certain aspects—if the focus is to address imaging or plasma levels of a given substance, scans and blood sampling (3) are conducted at baseline, when effects are expected, and after treatment of the attack. (**b**) Intensity of headache is recorded on a numeric rating scale from 0 to 10 (0, no headache; 5, moderate headache; 10, worst imaginable headache). Note the biphasic response, comprising an immediate headache followed hours later by a migraine-like headache. Adapted from: Ashina M et al. Human models of migraine—short-term pain for long-term gain. Nat Rev Neurol. 2017. doi: https://doi.org/10.1038/nrneurol.2017.137 [1]

5.2 Evidence of CGRP Involvement in Migraine Induction

The first evidence of CGRP's ability to induce migraine attacks was published in 2002 (Table 5.1) [8]. In a double-blinded, placebo-controlled, cross-over study, 12 patients with migraine without aura were infused with 2.0 μg/min CGRP over 20 min [8]. Three patients were excluded during the study due to side effects (hypotension causing pallor and palpations). Three of the remaining nine patients (33%), who completed the study, developed headache fulfilling the criteria for migraine without aura according to the International Classification of Headache Disorders [9]. A subsequent placebo-controlled study reported a migraine induction rate of 77% [10], while no patients developed migraine-like attacks after the placebo [8, 10]. Interestingly, open-label studies report similar induction rates at 63%–75% [11–13]. A likely explanation for the discrepancy between induction rates of the first CGRP provocation study [8], in comparison to the subsequent studies [10–13] (33% vs. average ~71%), is the inclusion of "Criteria 2" in the later modified criteria for experimentally induced migraine (Table 5.2). Moreover, the studies demonstrated that the induced attacks mimic the patient's usual attacks and are treatable by acute-migraine medication [10–13]. Administration of CGRP to healthy participants may induce headache, but no migraine-like attacks [14, 15].

Table 5.1 Overview of human headache models of CGRP

Study	Population	Design	Dose	Migraine attack (%)	Aura (%)	Comment
Lassen et al. (2002) [8]	Migraine without aura	Double-blinded, placebo-controlled, crossover	2.0 μg/min, intravenous	33[a]	NA	NA
Hansen et al. (2008) [23]	Familial hemiplegic migraine	Compared to healthy controls	1.5 μg/min, intravenous	22	0	CACNA1A and ATP1A2 gene mutations in patients
Hansen et al. (2010) [16]	Migraine with aura	Compared to healthy controls	1.5 μg/min, intravenous	57	28[b]	NA
Hansen et al. (2011) [24]	Familial hemiplegic migraine	Compared to healthy controls	1.5 μg/min, intravenous	9	0	Unknown mutation in patients
Asghar et al. (2011) [12]	Migraine without aura	Within-subject repeated measurements	1.5 μg/min, intravenous	75	NA	MRA, sumatriptan
Guo et al. (2016) [11, 27]	Migraine without aura	Within-subject repeated measurements	1.5 μg/min, intravenous	63	NA	Familial aggregation and premonitory symptoms
Christensen & Younis et al. (2018) [10]	Migraine without aura	Double-blinded, placebo-controlled, crossover	1.5 μg/min, intravenous	77	NA	Efficacy of erenumab treatment

(continued)

Table 5.1 (continued)

Study	Population	Design	Dose	Migraine attack (%)	Aura (%)	Comment
Younis & Christensen et al. (2019) [13]	Migraine without aura	Double-blinded, crossover	1.5 μg/min, intravenous	67	NA	Crossover with sildenafil
Iljazi et al. (2020) [77]	Chronic migraine	Single-arm, open-label	1.5 μg/min, intravenous	83	NA	NA

MRA Magnetic resonance angiography, *n* Number, *NA* Not applicable
[a]Did not apply "Criteria 2" of diagnostic criteria of migraine attacks in experimental human headache models
[b]One out of the four patients experienced aura without migraine-like headache

Table 5.2 Diagnostic criteria of migraine attacks in experimental human headache models

Headache must fulfill Criteria 1 or 2 [27, 78].	
Criteria 1	
Headache fulfilling the C and D criteria for migraine without aura in accordance with the International Classification of Headache Disorders [9]	C. ≥2 of following characteristics: i. Unilateral location ii. Pulsating quality iii. Moderate to severe pain intensity[a] iv. Aggravation or avoidance of routine physical activity[b] D. ≥1 of following: i. Nausea and/or vomiting ii. Photophobia and phonophobia
Criteria 2 Headache mimicking the patient's usual migraine attack, as described by the patient, and treatable with acute migraine medication[c]	

[a]Pain intensity is recorded on a numeric rating scale ranging from 0 to 10, where "0" denotes no pain and "10" denotes the worst imaginable pain. "4–6" reflects moderate intensity and "7–10" reflects severe intensity
[b]In-hospital phase: aggravation by cough; out-hospital phase: avoidance of routine physical activities (e.g. walking or climbing stairs)
[c]≥50% reduction of rated pain intensity of headache within 2 hours after administration of the acute antimigraine medication

Interestingly, CGRP induced aura only at a low frequency ($n = 4$) among 14 pure migraine with aura patients, while 57% of the participants experienced migraine attacks without aura for the first time [16]. Cortical spreading depression, a propagating wave of cortical neuronal depolarization followed by a wave of suppressed neuronal activity, is believed to be the underlying mechanism of migraine aura [17–20]. The relationship between CGRP and cortical spreading depression in migraine is yet unclarified [21, 22]. The lack of aura induction and attacks without aura (in patients suffering exclusively from migraine with aura) after CGRP administration suggests that CGRP acts more downstream of the aura phase.

Patients with familial hemiplegic migraine (FHM), a rare monogenetic subtype of migraine with aura, experience no significant headache or aura after CGRP [23, 24]. These data suggested that pathophysiologic pathways in FHM may differ

from those of common migraine subtypes [23–25]. In support, a preclinical study of rats with CACNA1 mutations demonstrated that the vasodilation of trigeminovascular dural arteries was lower after CGRP, as compared to controls, with no effect on the CGRP release in the trigeminovascular system [26]. This suggests desensitization or downregulation of the trigeminovascular CGRP receptor in patients with the FHM [26]. Interestingly, while migraine has a clear genetic component, patients with a high family genetic load do not appear to be more susceptibility to CGRP [27].

Taken together, the administration of CGRP demonstrates its consistent reproducibility of attacks in the human models. This supports that the susceptibility to CGRP is an innate quality of migraine, whereas CGRP's role in migraine aura is questionable. Moreover, genetic factors such as family load and genetic variants do not appear to contribute to the susceptibility to CGRP.

5.3 Mechanisms of CGRP-Induced Migraine Attacks

Intravenously administrated CGRP cannot easily pass the blood–brain barrier [28], suggesting that it acts peripherally. Possible sites of action of CGRP in the human models include receptors located in the vascular smooth muscle cells of the meningeal vessels, the surrounding primary trigeminal afferents and the trigeminal ganglion [29–34]. The most extensively studied aspect is the vascular effect.

A magnetic resonance angiography study demonstrated that CGRP dilated the middle meningeal artery (MMA) in migraine patients as well as healthy participants [12, 14]. The vasodilation of MMA was accompanied by CGRP-induced attacks in patients, occurring ipsilaterally to the pain side during unilateral attacks and bilaterally during bilateral headache [12]. Furthermore, MMA was constricted by sumatriptan, while alleviating the headache in patients [12]. Interestingly, investigations of patients during spontaneous attacks revealed no dilation of the extracranial arteries, but the time to scan from the onset of attacks was widespread in the study (range 15 min to 21 h) [35]. CGRP promotes neurogenic inflammation via vasodilation, mast cell degranulation, and release of proinflammatory mediators [36]. This may lead to modulation of the neuronal activity, consequently triggering a positive feedback loop causing sensitization of the peripheral trigeminal nociceptors [37–39]. The vasodilation during attacks might reflect an epiphenomenon of perivascular trigeminal sensitization and release of vasoactive substances, including CGRP [36, 37, 40].

The effect of the CGRP receptor antagonist, olcegepant, has been studied in the human model of CGRP [41]. When applied as pretreatment, olcegepant was capable of forestalling the headache-inducing effects of CGRP as well as side effects in healthy participants [28] with no *per se* hemodynamic effects, including the effect on cerebral blood flow [42]. A proof-of-concept clinical trial study demonstrated that olcegepant effectively aborted acute migraine attacks at rates comparable to sumatriptan, further solidifying the role of CGRP in migraine [43]. Interestingly, olcegepant *per se* does not constrict human cerebral arteries, as compared to sumatriptan [12, 42]. Altogether, the data suggest that gepants as non-constricting drugs,

in contrast to triptans, may abort migraine attacks likely by modulation of nociceptive transmission in trigeminal afferents [29–33, 44].

Premonitory symptoms, reported before head pain during migraine attacks, suggested an important role of central mechanisms in migraine initiation [45]. Studies using GTN as a migraine attack trigger reported premonitory symptoms before the onset of headache [45–48]. However, patients with migraine did not report premonitory symptoms after CGRP [11]. The question is whether CGRP, by acting on the trigeminal afferents, modulates sensory pain processing in the brain. Two studies investigated the possible central effect of CGRP in healthy participants [49, 50]. In the first functional magnetic resonance imaging (MRI) study, CGRP or placebo was intravenously administrated in combination with visual sensory input by checkerboard stimulation [49]. This study reported no blood-oxygen-level-dependent (BOLD) signal changes in the visual cortex (i.e., visual sensory processing) after CGRP infusion implying no changes in the brain activity [49]. Interestingly, sumatriptan did not affect visual sensory processing either after CGRP or placebo [49]. In the next functional MRI study, noxious heat stimulation in the V1 trigeminal area of the forehead increased the BOLD signal (i.e., increased activation) in insula and brainstem and decreased the BOLD signal (i.e., decreased activation) in the caudate nuclei, thalamus and cingulate cortex [50]. Given that these changes were observed only after CGRP infusion, it was suggested that CGRP might modulate the trigeminal nociceptive transmission, which in turn altered the activity in deep brain structures associated with pain processing [49, 50]. Interestingly, changes in the brain activity were reversed by sumatriptan [50].

A recent study reported increased perfusion in the dorsolateral pons during CGRP-induced attacks, which was previously shown during spontaneous attacks [51–55]. Increased perfusion was observed in the pons ipsilateral to the most painful side during attacks [51]. However, no corresponding increase in the glutamate levels was observed to support the hypothesis of increased abnormal pontine glutamate levels in migraine [51]. The exact implication of the pontine activation and its specificity to migraine is yet unresolved and needs further clarification.

Collectively, the data from the human models of CGRP suggest a peripheral site of action of CGRP in migraine induction. This includes cranial vasodilation as well as modulation of nociceptive transmission in the trigeminal afferents with subsequent modulation of the sensory processing in the central nervous system.

5.4 cAMP and cGMP Molecular Signaling Pathway

There are at least two recognized molecular pathways of migraine induction. One of the pathways is mediated by cyclic adenosine monophosphate (cAMP), which can be activated by CGRP via its specific receptor (Fig. 5.2). Another pathway is mediated by cyclic guanosine monophosphate (cGMP) and is intracellularly (i.e., more downstream than CGRP) activated by migraine triggers such as sildenafil and GTN [56, 57].

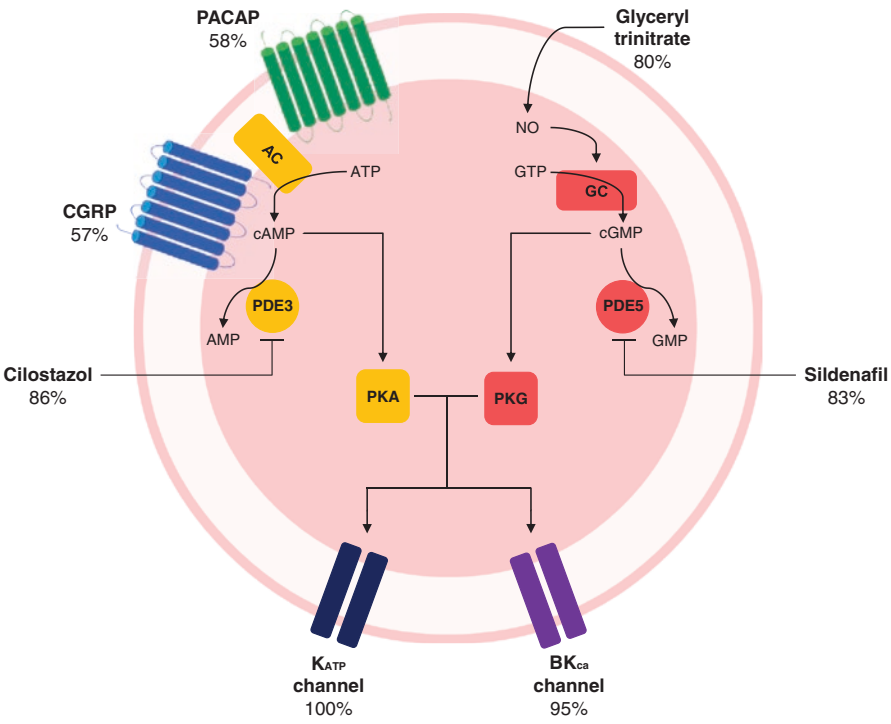

Fig. 5.2 Molecular signaling pathways of migraine induction. Calcitonin gene-related peptide (CGRP) binds to its extracellular CGRP-receptor leading to upregulation of cyclic adenosine monophosphate (cAMP), which is broken down by phosphodiesterase (PDE) 3 [36]. Cilostazol, a specific PDE-3 degradation inhibitor, induces migraine attacks at a higher induction rate than CGRP [10–13, 73, 74]. The disparity is likely ascribed to cilostazol's more downstream, intracellular effect as compared to CGRP and PACAP [34, 75]. Sildenafil, a selective inhibitor of PDE-5, upregulates cyclic guanosine monophosphate (cGMP), also by intracellular mechanisms [56], with a higher induction rate than CGRP. Migraine induction rates of both glyceryl trinitrate and sildenafil (via the cGMP pathway) are similar to cilostazol (cAMP pathway) [13, 73, 74, 76], suggesting that more downstream activation of pathways yield higher induction rates. In support, induction rate was higher when targeting the further downstream ATP-sensitive and large conductance calcium-activated potassium channels [60, 61]. Adapted from: Ashina M et al. [6] Migraine: disease characterisation, biomarkers, and precision medicine. Lancet. 2021; https://doi.org/10.1016/S0140-6736(20)32162-0. *ATP* Adenosine triphosphate, *AC* Adenylate cyclase, *PKA* Protein kinase A, *GTP* guanosine triphosphate, *GC* Guanylate cyclase, *PKG* Protein kinase G

The interaction between the cAMP and cGMP pathways was investigated in a forearm skin model of healthy participants [58]. Here, infusion of a nitric oxide-synthase inhibitor did not hinder the CGRP-induced vasodilation [58]. Additionally, provocation experiments in migraine patients demonstrated that GTN-induced migraine and vascular effects could not be prevented by the CGRP receptor antagonist olcegepant [59]. These data imply that the CGRP-mediated pathway does not interact directly with the cGMP pathway.

The question is whether these distinct molecular signaling pathways (cAMP and cGMP) can explain the clinical heterogeneity of migraine attacks. An alternative hypothesis is that the two different pathways merge within a common, more downstream denominator of the cAMP- and cGMP-signaling pathways, such as the ATP-sensitive potassium channels [60]. One study was conducted to investigate whether migraine induction, mediated via the cAMP and cGMP pathways, yields similar attacks within the same patients [62]. In a double-blinded, crossover design, migraine patients were randomly assigned to CGRP infusion and oral sildenafil on two experimental days [62]. The majority of participants (63%) developed migraine on both days with no difference in the clinical features. This suggests that clinical heterogeneity cannot be explained by distinct signaling pathways. Another important implication is that these two different signaling pathways may converge into a shared, more downstream target of which potassium channels have been suggested as the common denominator, such as the ATP-sensitive potassium channel [60, 62]. In support, a human model revealed a migraine induction rate of 100%, when patients were exposed to an ATP-sensitive potassium channel opener [60].

5.5 CGRP Models for Drug Testing

Human models can be used in early drug discovery programs. In a human dermal blood flow model, capsaicin was applied to the skin of the forearm [63]. Capsaicin produces neurogenic inflammation and vasodilation by local release of vasoactive mediators, including CGRP [63]. The human dermal blood flow model demonstrated that the CGRP receptor antagonist, telcagepant, was able to block the capsaicin-induced response (mediated by CGRP) establishing a pharmacokinetic/pharmacodynamic relation for the compound in humans [64]. The dermal blood flow model has also been applied to test the response of monoclonal antibodies [65, 66]. Erenumab, a monoclonal antibody targeting the CGRP-receptor, demonstrated that doses ≥70 mg could inhibit the dermal blood skin flow response by >90%, which corresponds well to the clinically used doses of 70 mg or 140 mg for the preventive treatment of migraine [65]. Likewise, galcanezumab, an anti-CGRP monoclonal antibody, was used in the model to provide a dose–response relationship that assisted in the selection of dose for phase II randomized controlled trials [66].

Another interesting concept is whether the human models of CGRP are useful to test the efficacy of new antimigraine drugs. Ideally, such models are conducted in healthy participants to increase the feasibility of testing as well as to avoid the many challenges in studies of patients including disinterest in having an attack induced in the first place. Accordingly, a human model of CGRP was applied to investigate whether the CGRP-induced mild headache in healthy participants was treatable by sumatriptan to validate such model [67]. A sustained intravenous infusion of 1.5 μg/min of CGRP for 2 h was administrated to healthy participants [67]. The rationale for the longer infusion was that it might induce more headache than after the 20-min infusions. However, this was not the case, as there was no difference in headache

between the two different infusion times [67]. Moreover, pretreatment with oral sumatriptan did not reduce the headache response as compared to placebo. The study concluded that a CGRP human model in healthy participants was not considered valid or applicable as a pragmatic model for drug testing of new treatment options [67]. An indirect gain from this study was the observation of gastrointestinal hyperactivity as a prominent adverse event following sustained infusion of CGRP, directing attention toward the possibility of obstipation as a side effect of CGRP-based migraine treatments [68].

5.6 Future Perspectives and Conclusion

Human experimental studies have provided crucial data on the role of CGRP in the migraine pathophysiology. However, several pivotal questions are still unanswered. One puzzling aspect is why about one-third of patients do not experience migraine attacks after CGRP infusion. Combined with the fact that not all patients respond to CGRP-based treatments [69], this suggests that CGRP is not the sole molecule involved in the migraine induction. This is supported by data showing that other signaling pathways can induce migraine attacks as well (Fig. 5.2). These observations generated the hypothesis that patients who develop provoked migraine attacks, following CGRP infusion, would benefit more from treatment with CGRP-based drugs. As such, varying sensitivity to CGRP between patients in the headache model may be used to predict patients' response to anti-CGRP treatment and clinical outcome. This aspect of sensitivity to CGRP as a predictor of efficacy to anti-CGRP treatment was explored in a pilot study of 13 migraine patients after treatment with erenumab [10]. The pilot study revealed that patients with an excellent effect to erenumab were highly susceptible to attacks after CGRP, suggesting that CGRP in human migraine models holds the potential as a biomarker for predicting anti-CGRP treatment efficacy [10]. This aspect should be investigated further in larger scale studies, including a larger sample of erenumab nonresponders.

The CGRP model can be expanded to other primary headache disorders [70]. In patients with cluster headache, intravenous administration of CGRP induced attacks in majority of patients during the active phase of their episodic cluster headache, while patients in remission developed no attacks [71], providing interesting clues to anti-CGRP treatment in cluster headache. In patients with persistent post-traumatic headache, CGRP induced headache exacerbation with migraine features in majority of patients. This finding suggests CGRP-targeted therapies as potential novel treatment option for persistent post-traumatic headache [72].

In the future, an optimized approach should consist of applying human models to screen different molecules and their physiological effects, whereas preclinical models should dissect these mechanisms in experiments, which would be impossible to conduct in humans.

Continuous and refined investigations of CGRP in the human headache models will continue to fill the gaps in our knowledge of CGRP-related mechanisms and lead to improved treatment regimes.

Glossary

Calcitonin gene-related peptide (CGRP) A vasoactive neuropeptide that modulates nociceptive transmission within the trigeminovascular system. There are two isoforms of CGRP, αCGRP and βCGRP. αCGRP is mainly in the central and peripheral nervous system, whereas βCGRP is mainly in the enteric nervous system.

Cilostazol A drug that inhibits phosphodiesterase 3 leading to accumulation of cyclic adenosine monophosphate.

Cyclic adenosine monophosphate (cAMP) A second messenger molecule able to activate protein kinase A (PKA).

Cyclic guanosine monophosphate (cGMP) A second messenger able to activate protein kinase G (PKG).

Glyceryl trinitrate (GTN) A nitric oxide donor.

Olcegepant The first selective small-molecule calcitonin gene-related peptide antagonist.

Phosphodiesterase 3 (PDE-3) An enzyme that degrades cyclic adenosine monophosphate to adenosine monophosphate.

Phosphodiesterase 5 (PDE-5) An enzyme that degrades cyclic guanosine monophosphate to guanosine monophosphate.

Sildenafil A drug that inhibits phosphodiesterase 5 leading to accumulation of cyclic guanosine monophosphate.

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Chapter 6
CGRP Antibodies for Animal Models of Primary and Secondary Headache Disorders

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6.1 Introduction

Migraine is ranked as the second cause of disability globally [1, 2]. Over the past two decades, calcitonin gene-related peptide (CGRP) has moved to the forefront as a critical neuropeptide involved in the pathophysiology of migraine and other headache disorders [3]. Despite the initial setbacks of several small molecule CGRP receptor antagonists (gepants) mostly due to liver toxicity [4], anti-CGRP antibodies targeting either the ligand or the receptor proved successful in the preventative treatment of episodic and chronic migraine [5, 6]. Most recently, several newer gepants have been approved for acute treatment of migraine [6, 7]. In addition, one of the CGRP antibodies, galcanezumab, has proven effective for preventing episodic cluster headache [6, 8]. Now, anti-CGRP antibodies are under consideration

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_6

for the treatment of secondary headache disorders, such as post-traumatic headache (PTH) and medication overuse headache (MOH) [6, 9–11].

Currently, there are three monoclonal antibodies against CGRP, galcanezumab (LY2951742), fremanezumab (TEV48125), and eptinezumab (ALD403), and one monoclonal antibody against the canonical CGRP receptor, erenumab (AMG 334), all of which have been approved by the Food and Drug Administration (FDA) [4]. As a multifunctional neuropeptide, CGRP participates in vasodilation, neurogenic inflammation, and nociception [12]. CGRP and its receptor components are distributed in peripheral and central tissues [13–15]. However, mechanisms underlying the role of CGRP in headache pathophysiology, and of anti-CGRP antibodies in its treatment, as well as their respective sites of action remain obscure.

Preclinical studies involving animal models of headache disorders are critical to the development of new drugs and understanding of their mechanisms. In this review, we aim to summarize preclinical studies investigating the role of CGRP (Table 6.1 and Fig. 6.1) and the efficacy of anti-CGRP antibodies in animal models of headache disorders (Table 6.2 and Fig. 6.1). Gepants and other CGRP receptor antagonists will not be discussed in detail here (see [16] for more information). A variety of animal models that mimic features of migraine have been developed and will be discussed in this review. Cluster headache is another primary headache involving CGRP. CGRP levels were increased in patients with cluster headache [17–19], and CGRP induced cluster headache attack in patients with cluster headache [20]. Galcanezumab was approved to treat episodic cluster headache by FDA [6, 8], but it was not effective for chronic cluster headache [21]. Sadly, animal models for cluster headache are limited and not ideal. Some cluster headache animal models overlap with migraine animal models [22]. Over the past decade, animal models for secondary headaches have been established, most notably for PTH and MOH. These secondary headaches often exhibit migraine-like features [23–25]. Therefore, the discussion of anti-CGRP antibodies will be mainly focused on migraine and secondary headaches PTH and MOH (Table 6.2).

6.2 Animal Models of Primary Headaches

6.2.1 Animal Models of Migraine

Migraine is a complex neurological disorder characterized by moderate or severe headaches with sensory alternations such as nausea, photophobia, and phonophobia. There is a variety of animal models of migraine which can be induced chemically (administration of NO donors, CGRP, inflammatory mediators), or by electrical or mechanical stimuli. Each model has its own set of advantages and limitations and mimics some of the symptoms observed in patients. Here we organized animal models of migraine into peripheral or central models according to injection sites/routes and stimulation sites. However, to be clear, the designation of migraine

Table 6.1 Summary of sites where CGRP levels were increased in different animal models for headache disorders

		CGRP		
Animal models		Sites of increased CGRP	Additional information for sites of increased CGRP	Refs.
Migraine				
Peripheral				
NTG	Acute	Plasma	• Samples: jugular vein; other peripheral blood vessels	[34–37]
		TG	• ↑ mRNA; protein	[38, 39]
		TNC	• ↑ CGRP-immunoreactive cell number	[33]
		Brainstem	• ↑ Protein	[34, 39]
	Chronic	Plasma		[45]
		TG	• ↑ mRNA; CGR P-immunoreactive cell number	[45, 46]
		TNC	• ↑ CGRP protein expression in fibers	[47, 48]
		Medulla-pons	• ↑ mRNA	[49]
		VN	• ↑ mRNA	[48]
Electrical stimulation of TG		Jugular vein	• ↑ Protein	[76–78]
		TG	• ↑ mRNA; protein	[79]
		TNC	• ↑ mRNA; protein	[79]
		Dura	• ↑ CGRP release	[80–82]
Activation of meninges	Electrical/ mechanical stimulation	Dura	• ↑ CGRP release	[84]
	TRP channels	TG and dura slices	• ↑ CGRP release by mustard oil, umbellulone	[87]
		Spinal cord slices	• ↑ CGRP release by umbellulone	
		TG neurons in culture	• ↑ CGRP release by Acrolein • No change of CGRP-immunoreactive cell number in TG and mRNA in dorsal root ganglion neurons in vivo	[88–90]
	Inflammation or decreased dural pH	TG	• ↑ CGRP expression in fibers induced by IS and CFA onto dura	[115]
		TNC	• ↑ Both CGRP and RAMP1 protein levels • Induced by repeated application of IS onto dura	[116]
		Dura	• ↑ CGRP release induced by H^+	[122]
		TG neuronsin culture	• ↑ CGRP release induced by H^+	[123]

(continued)

Table 6.1 (continued)

	CGRP		
Other			
CSD	Neocortical slices	• ↑ CGRP release	[157]
	TG	• ↑ CGRP-immunoreactive neurons • No change in CGRP mRNA levels	[158]
	Cortex	Multiple CSD • ↑ CGRP mRNA and protein levels • ↑ CGRP mRNA levels in frontal, motor, somatosensory, and visual cortices but not the cingulate cortex Single CSD • No change in CGRP mRNA levels	[159]
Secondary			
PTH	Brainstem including TNC	• ↑ Protein	[189]
	Meningeal layers	• ↑ CGRP immunoreactivity	[190]
	Plasma	• ↑ Protein	[191]
	TG	• ↑ Protein expression in each cell and total number of CGRP-immunoreactive cells • Even after tactile hypersensitivity had resolved 2 weeks after injury	[192]
	TNC	• ↑ CGRP protein levels with repeated closed head injuries > ↑ CGRP protein levels with a single injury	[193]
MOH	Spinal cord	• ↑ CGRP immunostaining induced by morphine	[199]
		• ↑ Capsaicin-evoked CGRP release, and CGRP immunoreactivity induced by morphine	[200]
	Dura	• ↑ CGRP-expressing dural afferent neurons induced by morphine • ↑ Even after tactile hypersensitivity resolved	[201]
	TG	• ↑ CGRP-expressing cells induced by triptans • ↑ Even after tactile hypersensitivity resolved	[202]
	Plasma but not in cerebrospinal fluid	• ↑ Protein levels induced by sumatriptan and bright light stress	[186]

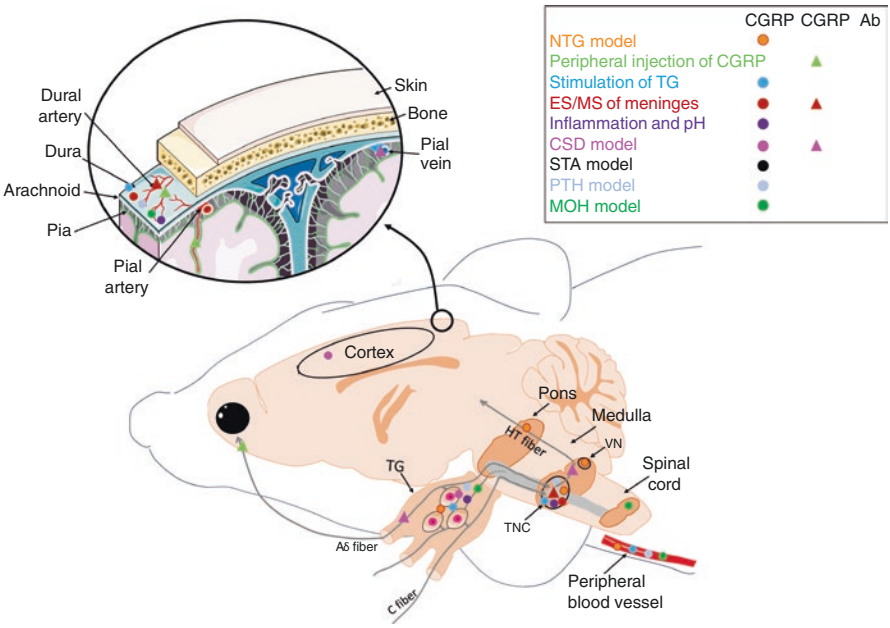

Fig. 6.1 *The sites of elevated CGRP levels and action sites of anti-CGRP antibodies in different animal models for headache disorders.* In most animal models, increased CGRP levels were observed in peripheral tissues (blood vessels, trigeminal ganglia (TG), dura) or the central nervous system trigeminal nucleus caudalis (TNC; the caudal part of the spinal trigeminal nucleus). Elevated CGRP was also reported in other central sites (cortex, pons, vestibular nucleus (VN), and spinal cord) in some headache models. Anti-CGRP antibodies most likely act in the periphery to modulate the peripheral nervous system, in particular trigeminal Aδ meningeal nociceptors and high threshold (HT) neurons in the spinal trigeminal nucleus, although it is not known if the latter occurs by direct or indirect effects. Actions at dural vessels are also likely to occur. *ES/MS* electrical or mechanical stimulation, *CGRP Ab* anti-CGRP antibody. From Servier Medical Art. Figure licensed under a Creative Commons Attribution 3.0 Unported License

models as being peripheral models does not at all mean to infer that the central nervous system (CNS) is not activated in these models. Conversely, we do not mean to infer that there are not any peripheral consequences in the central models.

6.2.1.1 Peripheral Models

Nitroglycerin-Induced Model

Nitroglycerin (NTG; or glyceryl trinitrate, GTN) is a highly lipophilic organic nitrate and a nitric oxide (NO) donor. NTG infusion was shown to cause migraine attacks in migraineurs [26–28] accompanied with an increase of plasma CGRP levels [28]. This discovery led to the development of NTG-induced animal models of migraine.

Table 6.2 Summary of effects and action sites of anti-CGRP antibodies in different animal models for headache disorders

	Anti-CGRP antibodies			
Animal models	Injection route	Effects	Action sites	Ref.
Migraine				
Peripheral				
NTG	I.p. 10 mg/kg, ALD405; mice	• ↓ NTG-induced chronic and acute extracephalic cutaneous hyperalgesia	• ALD405 acts peripherally, not centrally	[30]
	I.c.v. 10 μg, ALD405; mice	• Not ↓ NTG-induced extracephalic cutaneous allodynia		[52]
SNP	I.p., 10 mg/kg, ALD405; mice	• ↓ Facial allodynia Blocking effects significant in females than males		[53]
CGRP	I.p., 30 mg/kg, ALD405; mice	↓ CGRP i.p. induced • Light aversion • Grimace and squint		[62] [61]
	P.orb. 10 μL/60 pmol/site, mouse CGRP mAb, clone [4901]; mice	• ↓ Periorbital mechanical allodynia induced by CGRP (p.orb.)	• Peripheral terminals of trigeminal afferents	[63]
	I.v., 10 mg/kg, humanized CGRP mAb; rats	• ↓ Vasodilation of dural artery induced by CGRP (i.v.)	• Dural artery	[66]
	I.v., 30 mg/kg, fremanezumab; rats			[67]
	CGRP antiserum over cortical surface; rats	• ↓ Vasodilation of pial arterioles induced by CGRP over the cortical surface	• Pial arterioles	[68]
	I.d., anti-CGRP antiserum; rabbits	• ↓ CGRP potentiated histamine-induced edema	• Peripheral nerve terminals	[69]
	I.p., humanized CGRP Ab; mice	• ↓ I.p. CGRP-induced diarrhea (Ab3, 30 mg/kg) • ↓ CGRP-induced excrement (Ab6, 50 mg/kg)		[73]

	Anti-CGRP antibodies			
Electrical/ mechanical stimulation of meninges	I.v., 30 mg/kg, Fremanezumab; rats	• ↓ Spontaneous and dura stimulation-evoked HT neuron activities in spinal trigeminal nucleus	• Unclear whether direct or indirect effect	[85]
	I.v., 10 mg/kg humanized CGRP mAb; rats	• Not ↓ vasodilation of dural and pial artery induced by electrical stimulation	• Acute CGRP mAb injection cannot reach cranial vessels to block perivascular CGRP	[66]
	I.v., 10 mg/kg, CGRP mAb, muMab 7E9; rats	• ↓ Neurogenic vasodilatation of middle meningeal artery	• Middle meningeal artery	[86]
Other				
CSD	0.4 mM, CGRP Ab; mouse brain slices	• ↑ CSD latency		[160, 161]
	I.v., 30 mg/kg fremanezumab; rats with compromised BBB	• Not ↓ the induction, occurrence, or propagation of CSD • ↓ Propagation velocity and cortical recovery period of CSD • Similar to results from the isotype antibody		[162]
	I.v. 30 mg/kg, fremanezumab; rats	• ↓ The percentage of activation of Aδ but not C meningeal nociceptors	• CSD activates Aδ-HT and C-WDR pathways • Fremanezumab would act on Aδ-HT pathway	[163]
	I.v., 30 mg/kg, fremanezumab; rats	• ↓ Spontaneous or dura stimulation-evoked CSD-sensitive HT but not WDR neuron activities in spinal trigeminal nucleus		[85]
	I.v., 30 mg/kg, fremanezumab; rats	• Not ↓ dural artery dilatation and PPE induced by CSD • ↓ Pial vein dilatation by CSD	• Pial vein	[67]
STA rats	I.p., 10 mg/kg, ALD405; rats	• ↓ Cephalic hyperalgesia • Rapid-onset and long-lasting effects	• A peripheral site of action	[30]

(continued)

Table 6.2 (continued)

	Anti-CGRP antibodies			
Stress	I.p., 10 mg/kg, ALD405; mice	• Restraint stress • ↓ Facial allodynia		[53]
	S.c., 30 mg/kg, Fremanezumab; male rats	• Bright light trigger in MOH models • ↓ Cutaneous allodynia		[186]
Secondary				
PTH	I.p. 30 mg/kg, mouse CGRP Ab; male rats	• ↓ CHI-induced cephalic tactile hypersensitivity • ↓Subthreshold NTG-induced cephalic hypersensitivity	• Females showed longer phenotypes, increased responsiveness to triggers, and showed a poorer response to CGRP Ab than males	[195]
	I.p. 30 mg/kg, mouse CGRP mAb; female rats	• Not ↓ CHI-induced cephalic hypersensitivity • ↓ Cephalic hypersensitivity in response to subthreshold NTG		[196]
	I.p. 30 mg/kg, mouse CGRP mAb; male rats	• ↓ Cephalic hyperalgesia in more severe or repetitive CHI models		[197]
	I.p. 30 mg/kg, mouse CGRP mAb; male mice	Early and continuous CGRP mAb • ↓ Immediate cephalic and extracephalic hypersensitivity • ↓ Later sensitization induced by light		[198]
MOH	S.c., 30 mg/kg Fremanezumab; male rats	• ↓ Cutaneous allodynia induced by bright light/NO donor in sumatriptan/morphine MOH models		[186]

	Anti-CGRP antibodies			
CGRP Ab spreading test	I.v., 30 mg/kg, fluorescently-conjugated fremanezumab (Frema594); rats	Rats with uncompromised BBB: • Periphery: Intense labeling in dura, dural blood vessels, TG, C2 dorsal root ganglion, and autonomic ganglia • CNS: No labeling in the spinal trigeminal nucleus, thalamus, hypothalamus, or cortex Rats with compromised BBB: • Periphery: Similar to labeling in rats with uncompromised BBB • Labeling in the cortex, penetrating 100 um from the compromised BBB site	• Peripheral site of action	[153]
	I.v., 30 mg/kg, fluorescently-conjugated fremanezumab (Frema594); rats, mice	• In dural and pial blood vessels • In dura outside of blood vessels		[67]
	S.c. 4 mg/kg, [^{125}I] galcanezumab; rats	• Plasma ≫ dura mater > TG ≫ hypothalamus = spinal cord = prefrontal cortex = cerebellum		[152]

CGRP mAb Anti-CGRP monoclonal antibody

NTG is primarily used for studies on acute migraine-like behaviors and has been shown to produce hypersensitivity to pain, nocifensive behavior, and light aversion [29–33]. Similar to what was observed in patients, administration of NTG in animals also induced an increase in CGRP levels: in plasma [34–37], trigeminal ganglion (TG) [38, 39], trigeminal nucleus caudalis (TNC) [33], and brainstem [34, 39]. Moreover, some studies reported that NTG-induced increases in CGRP were accompanied by migraine-like behaviors, suggesting that there is a temporal association between behaviors and CGRP levels [33, 35, 36, 38, 40]. Besides, RAMP1-positive neurons were increased after NTG injection to rats [41]. More recently, a chronic migraine model was developed using repeated application of NTG [42–44]. Preclinical studies showed that repeated NTG infusion elicited nociceptive behaviors together with an increase in CGRP levels in plasma [45], TG [45, 46], TNC [47, 48], medulla pons [49], and vestibular nucleus (VN) [48]. As expected, the increase in CGRP mRNA levels in TG induced by repeated NTG administration was higher than that induced by a single injection of NTG in rats [45], which is consistent with the discovery that the plasma CGRP levels in the interictal period are higher in chronic migraineurs than in episodic migraineurs [50]. The increase of CGRP levels by NO donor might be through mitogen-activated protein kinase pathways [51]. Altogether, the models all indicate that CGRP is associated with NTG actions.

Several recent studies explored the efficacy of anti-CGRP antibodies on migraine-like behavior in the NTG model (Table 6.2). Christensen et al. showed that chronic and acute extracephalic cutaneous hyperalgesia provoked by repeated NTG in mice was prevented by pretreatment with ALD405 (intraperitoneal, i.p.), a humanized monoclonal CGRP antibody [30]. When the effects of ALD405 and olcegepant were compared by using the same experimental setting in this study, it was found that only acute, but not chronic extracephalic hyperalgesia induced by repeated NTG administrations was prevented by olcegepant. This suggests that ALD405 can be used as a preventative treatment for migraine, whereas olcegepant acts only acutely. Later, Christensen et al. demonstrated that intracerebroventricular (i.c.v.) injection of ALD405 to C57BL/6J mice was not able to prevent NTG-induced extracephalic cutaneous allodynia [52]. It suggests that ALD405 does not act centrally to alleviate migraine pain in this NTG model. However, this does not necessarily rule out a central site of action of drugs targeting CGRP signaling in migraine. The effect of i.c.v. ALD405 in other migraine-like behaviors or other migraine models should be tested in the future.

Recently, another NO donor, sodium nitroprusside (SNP) was used as a trigger to induce migraine-like symptoms in primed animals [53, 54]. Subthreshold dose of SNP administration elicited facial allodynia following dural CGRP administration in females [54] or repetitive stress [53]. The priming to SNP induced by repetitive stress was blocked by ALD405 (i.p., 10 mg/kg), which was significant in females and less apparent in males. The sex-dependent effects suggesting the sexually dimorphic role of CGRP and anti-CGRP antibodies in animal models priming to SNP. Recently, Zhang et al. showed that knockdown of CGRP in TG attenuated the increase of CGRP levels and activation of TNC and VN induced by repeated NTG

infusion [48]. More excitingly, it rescued the thermal hyperalgesia and vestibular dysfunction in this chronic migraine model. This suggests a potential effect of anti-CGRP antibodies in vestibular migraine patients.

Models Induced by Peripheral Injection of CGRP

The discoveries that plasma CGRP levels were increased in migraine patients [55] and intravenous (i.v.) injection of CGRP induced migraine attacks in migraineurs [56] facilitated the development of another animal model of migraine by peripheral injection of CGRP.

Light aversion in mice is a surrogate for photophobia in patients which is presented in 80–90% of migraineurs [57, 58]. It is identified as the most bothersome symptom for migraine patients [59]. Grimace or squint is an indicator for spontaneous pain [60, 61]. Studies by the Russo group showed that i.p. CGRP induced light aversion [62], grimace, and squint [61] in mice, which were prevented by ALD405 pretreatment (i.p., 30 mg/kg). Another group found that periorbital mechanical allodynia induced by CGRP (subcutaneously, s.c.) can be rescued by a mouse monoclonal anti-CGRP antibody [63]. Both compounds were injected into periorbital areas of C57BL/6J mice [63], suggesting actions of CGRP and the anti-CGRP antibody on peripheral terminals of trigeminal afferents. Intra-ganglionic injection of CGRP produced thermal orofacial hyperalgesia [64] and periorbital mechanical allodynia, light sensitivity, and anxiety in rats [65]. Dural application of CGRP elicited periorbital allodynia only in female mice and rats [54]. In addition, dural application of IL-6 and intracisternal administration of BDNF only primed female animals to subthreshold dural CGRP, and dural CGRP primed females to pH 7.0 or subthreshold SNP [54]. This finding suggests the involvement of dural CGRP in migraine pain and ability of dural CGRP to sensitize females to non-noxious migraine triggers. Besides, it highlights the dura mater as one of the action sites of CGRP and possible sexual dimorphism in drug responses.

There has been an ongoing debate for years on whether mechanisms underlying migraine are vascular or neuronal. The answer still remains unclear, but a cranial vascular mechanism cannot be ignored. Therefore, when effects of anti-CGRP antibodies on animal models of migraine are discussed, it is important to consider cerebral vascular aspects. Work by Juhl et al. showed that an anti-CGRP antibody (i.v.) significantly reduced the vasodilation of dural artery induced by CGRP (i.v.) in rats [66], which is consistent to results in a recent study conducted by Burstein laboratory using fremanezumab (i.v.) in rats [67]. When cerebral cortical surfaces of rats were suffused with CGRP antibody serum, the vasodilation of rat pial arterioles induced by CGRP over the cortical surface was inhibited [68]. Moreover, CGRP potentiated histamine-induced edema in rabbits, which was impeded by pretreatment of anti-CGRP antiserum (intradermal, i.d.) [69]. Results suggest not only the ability of CGRP to enhance histamine-provoked protein plasma extravasation (PPE) but also actions of anti-CGRP antiserum in the peripheral nerve terminals.

Gastrointestinal symptoms occur in 22% migraine patients [70]. CGRP infusion caused gastrointestinal problems (e.g., diarrhea) in healthy volunteers [71], and 3–4% patients reported constipation as an adverse effect after erenumab [72]. Our team reported that CGRP (i.p.) induced diarrhea, which was blocked by anti-CGRP antibodies (i.p.) in mice [73]. Further delineation of whether gastrointestinal symptoms will be beneficial or considered as adverse effects from anti-CGRP antibodies will provide better health care for migraineurs with/without gastrointestinal symptoms.

Models Induced by Electrical Stimulation of TG

TG is activated during migraine [74]. It is believed that trigeminal nerves on dural and pial arteries are likely to be involved in the initiation of migraine pain [75]. Thus, the stimulation of TG has been applied to model migraine in preclinical research. TG cell bodies are the main source of CGRP. Preclinical studies demonstrated that electrical stimulation of TG enhanced CGRP levels in jugular vein [76–78], TG [79], TNC [79], and the release of CGRP from nerve fibers in the dura [80–82]. Electrical stimulation of TG increased the cerebral blood flow [77] and activated the trigeminal complex [83]. Given its importance in migraine pathophysiology, it is necessary to investigate the effects of anti-CGRP antibodies in this model.

Models Induced by Activation of Meninges

Electrical or Mechanical Stimulation of Meninges

Meningeal vasculature is innervated by trigeminal nerves. Meningeal sensory afferents pass through TG to enter the trigeminocervical complex, which relays signals to other CNS regions, including those involved with pain. Just as described in the TG model, trigeminal nerves on dural and pial arteries may contribute to the onset of migraine pain [75]. Therefore, investigators attempted to perform meningeal electrical or mechanical stimulation in animals to model migraine.

Electrical stimulation of dura induced CGRP secretion from dural nerve fibers in rats [84]. Effects of anti-CGRP antibodies on the activity of trigeminovascular neurons and meningeal arteries were investigated. Melo-Carrillo et al. showed that fremanezumab (i.v.) inhibited spontaneous and dura stimulation-evoked high threshold (HT) neuron activities in the spinal trigeminal nucleus of rats [85]. Dural and pial artery dilatations induced by electrical stimulation in a cranial closed window of rats were not blocked by an anti-CGRP antibody (i.v.) reported by the Jansen-Olesen group [66]. The authors presumed that acute injection of the anti-CGRP antibody cannot reach the cranial vessels to block perivascular CGRP after electrical stimulation. Interestingly, another study from the Shelton group showed that an anti-CGRP antibody (i.v.) inhibited neurogenic vasodilatation of middle meningeal artery induced by electrical stimulation in the cranial window of rats

[86]. The discrepancy might arise from the different antibodies used, different setup of electrical stimulus, and different time points chosen. Based on the finding that the anti-CGRP antibody only manifested significant effects 90–120 min after injection in the later study [86], the Shelton group thinks that the short time interval used by the Jansen-Olesen group [66] is not sufficient for antibody to penetrate the cerebral vessel wall [86].

Transient Receptor Potential (TRP) Channel Activation

TRP channels are a family of cation channels. They respond to a variety of stimuli related to migraine and are expressed on the meningeal nociceptors. Those facts make TRPV a potential candidate in the migraine pathophysiology.

Stimuli for TRPA1 include mustard oil and environmental irritants, umbellulone, and acrolein. Studies showed that mustard oil and umbellulone induced CGRP release in animal slices of TG and dura mater. Umbellulone also induced CGRP release in spinal cord slices and these effects were TRPA1-dependent [87]. Exposure of TG cultured neurons to acrolein stimulated CGRP release [88] while no change of CGRP levels in TG and dorsal root ganglion of acrolein-exposed rats compared to room air-exposed rats [89, 90]. Preclinical studies showed that dural application of mustard oil or umbellulone induced tactile extracephalic and cephalic allodynia and decreased exploratory rearing behavior [91, 92]. These effects were prevented by a TRPA1 antagonist [91]. The exploratory rearing behavior decreased by dural mustard oil was blocked by an antimigraine medication sumatriptan.

In addition to TRPA1, other TRP channels have been recruited for migraine models. These include capsaicin as an activator of TRPV1 [93], hypotonic solution and 4α-PDD as activators of TRPV4 [94], and icilin as an activator of TRPM8 [95, 96]. Application of these agents to the dura produced facial and hindpaw allodynia. The situation for TRPM8 is complex since activation of TMPM8 by menthol was antinociceptive [96, 97].

As an alternative to dural applications, several TRP agonists have been applied by inhalation and other routes. Inhalation of acrolein induced migraine-like behaviors (e.g., cephalic allodynia, anxiety, and scent preference) compared to rats exposed to room air [98]. Nasal administration of umbellulone, mustard oil, acrolein and capsaicin induced meningeal vasodilation [87, 88, 99], which was blocked by CGRP receptor antagonists [87, 88]. I.v. mustard oil or umbellulone also increased dural blood flow, which was blocked by a CGRP receptor antagonist (i.v.) [87]. Injection of complete Freund's adjuvant (CFA) into trapezius muscle primed rats to the oil extract prepared from California Bay leaf (where umbellulone is isolated) [100]. The rats with CFA treatment and inhalation of California Bay leaf extract displayed nociceptive behavior compared to control rats [100]. Here, neck muscle inflammation induced by neck injection of CFA is assumed to facilitate sensitization of trigeminal neurons [100] considering neck pain is a migraine trigger or a frequent accompaniment of migraine [101–104]. Altogether, TRP channel activation induces CGRP release and mimics migraine phenotypes. To our knowledge, the effects of anti-CGRP antibodies on these models have not been identified.

Considering the possible limitations of blocking TRP channels (e.g., dysfunction of thermosensation via TRPV1 blockage during daily life), it will be worthwhile to detect the effects of anti-CGRP antibodies on these models.

Dural Inflammation and Decreased Dural pH

Dural inflammation was considered a critical role in migraine [105–107]. TG activation provokes CGRP release [80]. Subsequently, CGRP activates dural mast cells. It leads to mast cell degranulation and then the release of a wide variety of inflammatory mediators [12, 108]. Thus, dural administration of inflammatory mediators is regarded to model migraine-like pain phenotypes.

Dural administration of inflammatory soup (IS) [92, 109] or orofacial treatment of CFA [110] elicited cephalic allodynia in animals. A mixture of IS and capsaicin onto dura induced robust nociceptive behavior compared to dural capsaicin-treated mice, which was alleviated by sumatriptan and a CGRP receptor antagonist [111]. Inflammatory mediator solution [112] or IL-6 onto dura [92, 113, 114] elicited extracephalic and cephalic allodynia in animals. The Dussor group showed that dural IL-6 application primed mice/rats to the subthreshold migraine trigger SNP, pH 6.8 or 7.0 solution [92, 114], and subthreshold dose of CGRP in female rats [54].

In addition to the migraine-like behavior induced by dural inflammation, it also changed CGRP levels (Table 6.1). Dural application of IS or CFA into rats induced an increase of CGRP-immunoreactive fibers in TG [115]. In a rat model of recurrent migraine established by repeated application of IS onto dura, periorbital and hind paw allodynia together with a significant enhancement on CGRP protein levels in TNC were observed [116]. Moreover, meningeal application of inflammatory mediators such as IL-1β and IL-6 modulated rat meningeal nociceptors [113, 117]. It seems that a positive feedback loop between meningeal nociceptors, CGRP and inflammation exists, which sustains the activation and sensitization of meningeal nociceptors [107, 118].

Besides the release of inflammatory mediators, degranulation of mast cells may acidify the local environment [119]. Dural administration of pH 6.0 solution provoked headache-like behavior in mice [92]. Co-application of subthreshold dose of mast cell mediators including inflammatory mediators with pH 6.6/6.8 onto dura led to facial and hindpaw allodynia compared to the application of them separately [120]. Repetitive stress primed mice to dural pH 7.0 to exhibit facial hypersensitivity but not to pH 7.4 [53]. It is believed that acid-sensing ion channels are related to dura pH change [93, 120, 121]. H^+ evoked CGRP release in dura [122], and in TG cultured neurons via ASIC3 [123]. The change of pH excited dural afferents [93], where CGRP is released. Based on these findings and combined with the inflammation feedback loop mentioned above, it is speculated that there is a cycle for meningeal nociceptors, CGRP release, mast cell degranulation, inflammation, and acidification in the migraine pathophysiology [120]. Thus, blocking any components including using anti-CGRP antibodies might show benefits to migraine-like pain behavior induced by inflammatory mediators or H^+.

6.2.1.2 Central Models

Models Induced by Central Injection of CGRP

CGRP and its receptors are not only distributed in the peripheral tissues but also widely expressed in the central tissues [13–15]. CGRP is localized in cell bodies in the hypothalamus, amygdala, hippocampus, ventromedial nucleus of the thalamus, and parabrachial nucleus; fibers in the thalamic areas, amygdala, hypothalamus, periaqueductal gray, and parabrachial nucleus. CGRP-binding sites include the hypothalamus, amygdala, ventrolateral thalamic areas, and cerebellum [13–15, 124, 125].

Central injection of CGRP into animals displayed migraine-like behaviors. I.c.v CGRP into wildtype C57BL/6J mice induced light aversion to very bright light [62, 126]. Plantar hyperalgesia was observed in response to thermal stimuli after i.c.v. CGRP infusion, although whether it was exhibited in C57BL/6 J or AKR mice was not clear [127] and intrathecal (i.t.) administration of CGRP [128]. Rats produced a significant plantar hyperalgesia to mechanical stimuli after i.t. CGRP [129]. Nestin/hRAMP1 transgenic mice express hRAMP1 selectively in nervous tissues [130] and had increased sensitivity to CGRP manifested by light intensities and drug doses. Dim light was sufficient for CGRP (i.c.v.) to induce light aversion in nestin/hRAMP1 mice when bright light was required for wild-type C57BL/6J mice [62, 126, 131, 132]. Injection of a low dose of CGRP (i.t.) into nestin/hRAMP1 mice induced mechanical nociception while littermate controls required a higher dose [133]. Taken together, these data suggest that CGRP can act on the CNS to induce migraine-like behaviors.

Different brain regions are responsible for different functions. Researchers tried to reveal the role of CGRP in a specific brain region. Our team showed that CGRP delivery to the posterior thalamic area induced light aversion without anxiety-like behaviors in mice [134]. CGRP administration into the latero-capsular division of the central nucleus of the amygdala (CeLC) exacerbated pain responses demonstrated by a significant increase in vocalization and hind limb hyperalgesia to mechanical stimuli in rats [135]. Conflicting results occurred as studies showed that CGRP infusion into the central nucleus of the amygdala (CeA) or the basolateral nucleus amygdala (BLA) of rats was antinociceptive [136, 137]. The disparate data might attribute to different amygdala regions or amygdala pathways targeted. CGRP also induced antinociceptive effects when applying to the nucleus accumbens, nucleus raphe magnus, and periaqueductal gray [138–141]. Recently, the Palmiter group found that inactivation of CGRP-containing neurons in the parabrachial nucleus blunted pain signals in mice [142]. Those data suggest the site specificity for CGRP effects on pain modulation. Moreover, central CGRP was able to modulate anxiety, depression, and fear memory in animals, which can be involved in the emotional and cognitive components of pain [127, 142–150]. I.c.v. CGRP antiserum promoted the extinction of avoidance responses in rats, suggesting the impairment of learning and memory [151]. Altogether, CGRP in the CNS plays a role in multidimensional aspects of pain. Even though preclinical evidence showed that

current anti-CGRP antibodies have limited ability to cross BBB when injected peripherally [152, 153], clinical efficacy arising from possible central actions cannot be excluded. More investigations are needed to reveal the function of CGRP in the CNS, which will help justify the necessity or not for the development of brain-penetrant anti-CGRP therapeutics.

6.2.1.3 Other Models

Models of Cortical Spreading Depression (CSD)

CSD is an intense wave of depolarization followed by a depression in neuronal activity which propagates slowly across the cortex. It is hypothesized that CSD is the trigger of migraine aura [154]. Some migraineurs with aura showed aura symptoms after i.v. CGRP [155]. The links between CGRP and CSD were described in a review by Close et al. [156]. Here, we will mainly discuss the involvement of anti-CGRP antibodies in CSD animal models and updated findings (Table 6.2). In rat brain slices, CGRP failed to evoke CSD [157]. However, after inducing CSD in vitro by elevation of potassium concentrations in rat neocortical slices, endogenous CGRP was released in the cortical tissues, and CGRP receptor antagonists had a dose-dependent inhibitory effect on CSD [157]. This inhibitory effect induced by the CGRP receptor antagonist was partially reverted by adding exogenous CGRP [157]. It was discovered that CGRP-immunoreactive neurons but not CGRP mRNA levels in TG were increased 2 h post-CSD induction by KCl onto the parietal cortex of rats [158]. In contrast, another study showed that CGRP mRNA and protein levels in the cortex were increased 24 h after multiple CSD events, but CGRP mRNA levels were not increased after a single CSD event [159]. Importantly, upregulation of CGRP mRNA levels was observed in frontal, motor, somatosensory, and visual cortices but not the cingulate cortex, suggesting a regional specificity [159]. Later, Close et al. proposed a model where CSD increased peripheral and central CGRP levels and maintained them via vascular-neural communication [156].

Preclinically, effects of anti-CGRP antibodies on CSD events were reported (Table 6.2). Anti-CGRP antibody treatment was shown to elevate KCl-induced CSD latency in a mouse brain slice model [160, 161]. The Burstein group reported that pretreatment of fremanezumab (i.v.) did not inhibit the induction, occurrence, or propagation of CSD induced by pinpricking occipital cortex, but decreased the propagation velocity and cortical recovery period in rats with compromised blood–brain barrier (BBB) [162]. But in the same experiment, the isotype antibody used as a control showed similar effects, which makes interpreting the effect of fremanezumab on CSD difficult. Aside from a possible immunoglobulin-mediated effect, another explanation for the similarity of the results between fremanezumab and its isotype could reside in the antibody's action site. The Burstein group later measured how far fremanezumab spread in the rat brain after the BBB was compromised and found that fluorescently-conjugated fremanezumab (i.v.) penetrated into the cortex as far as 100 μm from the compromised BBB site [153]. The limited diffusion of

fremanezumab getting into the brain suggests a peripheral site of action. As a result, the question remains whether CSD can be inhibited by a brain-penetrant anti-CGRP antibody.

Melo-Carrillo et al. demonstrated that the percentage of activation of Aδ but not C meningeal nociceptors by pinpricking cortex-induced CSD was inhibited by pretreatment of fremanezumab (i.v.) to rats [163]. The same group reported that fremanezumab (i.v.) blunted the spontaneous or dura stimulation-evoked CSD-sensitive HT neuron activities but not wide dynamic range (WDR) neuron activities in the spinal trigeminal nucleus where CSD was induced by pinpricking the visual cortex of rats [85]. Based on these findings, a mechanism underlying fremanezumab's action for migraine prevention was proposed in which CSD activates Aδ-HT and C-WDR pathways. But only the Aδ-HT pathway would be CGRP dependent and that would be where fremanezumab would act. Aside from neuronal mechanism underlying fremanezumab's action, the dural artery dilatation and PPE induced by CSD were also investigated but were not affected by fremanezumab (i.v.) injection into rats [67]. Unexpectedly, the authors observed an inhibition of CSD-induced dilatation of pial veins by fremanezumab in the same study [67]. The mechanism underlying this observation is unknown, but three hypotheses were proposed. First, CSD activates postganglionic parasympathetic neurons in the sphenopalatine ganglion, and then modulates pial veins [164]. Given that the distribution of CGRP immunoreactive fibers [165] and fluorescently-conjugated fremanezumab [153] in the sphenopalatine ganglion, fremanezumab may affect pial veins by modulating postganglionic parasympathetic neurons. Second, fremanezumab reduces the dilatation of pial veins via modulating the wall of the pial vein intraluminally. Third, the authors make an assumption that CSD in this experiment evokes CGRP release, which might be transported to the paravascular space surrounding pial veins via glymphatic system. If the limited amount of fremanezumab crossing BBB is near the pial vein, they might be sufficient to block CGRP around pial veins [67]. This puzzling but exciting finding needs further investigation. In conclusion, these discoveries suggest that modulation of peripheral Aδ nociceptors and central HT neurons, but neither dural arteries dilatation nor PPE, is more likely to contribute to the therapeutic effect of fremanezumab on migraine. To our knowledge, there are no preclinical studies investigating the effects of anti-CGRP antibodies on CSD-induced behaviors such as facial and plantar allodynia, and anxiety-like behavior [166, 167].

The Spontaneous Trigeminal Allodynia (STA) Model

The STA rat model is an idiopathic strain presenting sustained cutaneous cephalic hypersensitivity and is presumed as a model of chronic migraine [168]. The advantage of this model is that the cutaneous hyperalgesia is spontaneous and does not resolve with time compared to chemical or electrical stimulation. ALD405 (i.p.) effectively reversed cephalic hyperalgesia, with a rapid-onset and a long-lasting effect in STA rats [30]. The rapidity of onset suggests a peripheral site of action of ALD405.

Models Induced by Sleep Deprivation and Stress

Sleep is reported as one of the most common triggers for the primary headache disorders [169]. In terms of migraine, there is a significant correlation between sleep duration and migraine frequency [170]. Psychological sleep interventions are able to reduce the headache frequency and intensity [171]. Many preclinical studies demonstrated that sleep deprivation affected pain perception, which was summarized in a recent review [172]. For example, paradoxical sleep deprivation decreased the pain threshold to thermal stimuli in rats [173]. Using chemogenetic and optogenetic strategies to selectively activate CGRP-expressing neurons in the parabrachial nucleus led to wakefulness [174]. It will be very intriguing to examine the impacts of anti-CGRP antibodies on the animal models induced by sleep deprivation.

Stress, either physical or psychological, is one of the most common triggers for primary headache disorders [169, 175]. A temporal correlation between daily stress and migraine activity was reported [176]. Preclinical studies demonstrated that stress (water, food, isolation, sound, etc.) produced higher pain sensitivity [177–182]. Familial hemiplegic migraine model, *Cacna1a* mutant mice showed migraine-related head pain more frequently than the wild-type mice triggered by restraint stress [183]. Mast cell degranulation via corticotropin-releasing factor triggered by stress might be one of the mechanisms for stress-induced pain [184]. NTG increased pain responses induced by chronic immobilization stress in rats [185]. As mentioned above, restraint stress primed animals to subthreshold dose of SNP or small change of pH to induce pain responses. ALD405 (i.p.) was able to block pain responses in stress-primed animals following SNP treatment [53]. This suggests that stress might influence migraine pain in response to future migraine triggers. Bright light stress provoked an increase of plasma CGRP levels and cutaneous allodynia in MOH rat models induced by sumatriptan. This pain behavior was blocked by fremanezumab (30 mg/kg, s.c.) [186]. The details will be further deliberated in the MOH animal model. Considering that it is a common trigger in migraine, it is important to explore the mechanism of how stress contributes to migraine and the effects of anti-CGRP antibodies on different stress-induced models.

6.3 Animal Models of Secondary Headaches

6.3.1 Post-Traumatic Headache (PTH)

The International Classification of Headache Disorders (ICHD) defines PTH as a secondary headache occurring within 7 days of traumatic brain injury or occurring upon regaining consciousness after trauma [187]. Because PTH shares clinical characteristics of primary headaches such as migraine [23, 24], the potential role of CGRP in its pathophysiology, as well as the possible efficacy of CGRP targeting drugs in its treatment are at the forefront of PTH research. While diverse

preclinical models of traumatic brain injury exist [188], only a few of those have been used to study PTH, most of which have shown an implication of CGRP (Table 6.1). In brainstem tissues that include TNC, CGRP protein levels were elevated over weeks after controlled cortical impact (CCI) injury compared to craniotomy only animals, and this elevation was associated with an increased tactile hypersensitivity [189]. CGRP immunoreactivity was found in the meningeal layers post-injury in that same model, while they remained negligible in controls [190]. CGRP protein levels were also increased in plasma in rats [191], and in TG in a closed head impact mouse model (CHI, commonly known as the weight-drop model) [192]. Interestingly in the latter study, CGRP levels remained elevated after the symptom of tactile hypersensitivity had resolved 2 weeks after injury; at that time however, animals were sensitized to normally non-noxious doses of the migraine trigger NTG [192]. Using repeated closed head injuries, another study showed that animals displayed enhanced reduction in trigeminal thresholds associated with a greater increase in the CGRP protein levels in TNC than the ones observed with a single injury [193].

Preclinical attempts to alleviate PTH symptoms using CGRP targeting drugs have been very encouraging (Table 6.2). The CGRP receptor antagonist MK8825 decreased trigeminal allodynia and reduced photosensitivity in the CCI group compared to vehicle-treated animals [190]. Our team found that CHI mice could exhibit cephalic and extracephalic hypersensitivity in response to non-noxious CGRP [194]. The Levy group conducted several PTH studies with preclinical models using different injury paradigms. In a mild CHI model using a weight drop device in male rats, chronic administration of an anti-CGRP antibody starting immediately after injury and every 6 days subsequently attenuated CHI-induced cephalic tactile hypersensitivity and prevented subsequent subthreshold NTG-induced tactile hypersensitivity [195]. The same group conducted similar experiments on female rats in a subsequent study and showed that females displayed longer phenotypes, increased responsiveness to subsequent triggers, and a poorer response to anti-CGRP antibody treatment [196]. This suggests a sex difference in PTH-like pain, and it is possibly due to a greater involvement of CGRP signaling in males when compared to females in response to NTG. Later, the Levy group developed other methods to induce more severe or repetitive CHI models [197]. The results showed that both models displayed prolonged cephalic and extracephalic hyperalgesia in response to mechanical stimuli compared to the mild CHI model. But only cephalic hyperalgesia was alleviated by early and sustained administration of an anti-CGRP antibody to male rats [197]. The failure in extracephalic hyperalgesia suggests additional factors are involved. Another study used a different pattern of administration of the anti-CGRP antibody in male mice following injury by a weight drop. It was discovered that early and continuous CGRP blockade is necessary to alleviate both immediate tactile hypersensitivity (cephalic and extracephalic) and later sensitization using light as a trigger [198]. This study implies that the anti-CGRP antibody would not be able to alleviate PTH symptoms if the early window of administration has closed [198].

6.3.2 *Medication Overuse Headache (MOH)*

The repetitive or excessive use of headache therapies can lead to MOH in predisposed patients [25]. Some drugs such as triptans, opioids, ergotamines, and barbiturates, have significantly more potential to induce MOH [25]. Because MOH is linked and shares clinical characteristics with migraine [25], the potential involvement of CGRP as well as the possibility that anti-CGRP drugs could relieve MOH-induced symptoms are being investigated (Tables 6.1 and 6.2). Preclinical studies have shown that morphine exposure increased CGRP-like immunostaining [199] and evoked CGRP release in the spinal cord [200]. CGRP-expressing dural afferent neurons were increased following morphine-induced MOH [201]. Similarly, persistent exposure of rats to triptans induced an increase in CGRP-expressing cells in TG [202]. Interestingly, these two studies showed that this elevation persisted even after tactile hypersensitivity had resolved [201, 202]. Repeated application of sumatriptan evoked a significant increase of CGRP levels in plasma but not in cerebrospinal fluid after bright light stress [186].

Only a couple of preclinical studies have investigated the efficacy of CGRP targeting drugs on MOH. The triptan-induced cutaneous allodynia can be abolished by administration of a CGRP receptor antagonist [202]. Finally, administration of an anti-CGRP antibody (s.c.) is able to reverse cutaneous allodynia induced by both sumatriptan and morphine, couples with subsequent triggers such as bright light or NO donor [186].

6.4 Conclusion and Future Directions

In general, current preclinical studies show that anti-CGRP antibodies are likely to act on trigeminal neurons. The site of action is likely to involve a pathway involving Aδ meningeal nociceptors and HT neurons in the spinal trigeminal nucleus, although actions on meningeal vessels and cell bodies within the TG may also be contributory (Table 6.2 and Fig. 6.1). Importantly, CGRP levels are increased in many headache models in both the periphery and within the CNS (Table 6.1 and Fig. 6.1). Hence, models that target these central sites of action should be further developed. Given that CGRP in different brain regions regulates different dimensions of pain, CGRP blocking medications acting on CNS might show benefits for the alleviation of headache disorders. In particular, the cerebellum is the region with the highest CGRP binding activity [203] and contains CGRP and CGRP receptors [15, 204, 205]. It will be interesting to delve deeper into the role of cerebellar CGRP in migraine. Likewise, none of the current models distinguish between the two CGRP receptors. Since the peptide amylin can bind to one of the CGRP receptors [206], this opens the door for amylin studies using the preclinical models. Identifying specific effects of amylin/CGRP in migraine-like behaviors using anti-CGRP antibodies may lead to better understanding of CGRP actions in migraine.

Acknowledgments The authors thank members of the lab for their comments. This work was supported by grants from the NIH (NS075599; NS113839) (A.F.R.). The contents do not represent the views of VA or the United States Government.

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Chapter 7
Galcanezumab

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7.1 Therapeutic Indication

Galcanezumab, formerly named LY2951742, is a humanized IgG4 monoclonal antibody, which was developed for the prevention of migraine and cluster headache [1]. Across several countries, the substance is approved for the prevention of migraine in patients with at least four migraine days per month [2]. It has also been licensed for the prevention of episodic cluster headache by some authorities such as the Food and Drug Administration (FDA) in the United States, but not by the European Medicines Agency (EMA) [3]. Galcanezumab failed to show efficacy in a study for the prevention of chronic cluster headache. It is therefore not indicated in this disease [4].

7.2 Mode of Action

Galcanezumab binds to the calcitonin gene-related peptide (CGRP) molecule with a high affinity (K_D = 31 pM) and a specificity of >10,000 fold compared to related peptides [1]. Due to this high affinity, the substance is able to bind more than 99% of free CGRP in an experimental setup, which is thought to resemble the human situation [1]. The binding to CGRP leads to a newly formed Galcanezumab–CGRP complex. This molecule has a change in its structure, which inhibits the anchoring on the CGRP receptor and thereby accounts for its efficacy in migraine prevention [1]. While the biological activity of CGRP is blocked, the CGRP receptor is not [1]. Whether this has any advantageous effect remains to be determined. The binding of Galcanezumab to CGRP is slowly reversible. It is hypothesized that any free

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_7

molecule of CGRP will be picked up by a free molecule of Galcanezumab [1]. Furthermore, the manufacturer of Galcanezumab assumes that the concentration of CGRP in human tissues increases under treatment with Galcanezumab due to experimental data (which are not published in a peer review manuscript). The clearance of the Galcanezumab–CGRP complex is slower than the physiological clearance of CGRP alone [1]. Due to its character of an antibody with a molecular weight of 150 kDa, Galcanezumab is supposed to act in migraine prevention without crossing the blood–brain barrier, which may result in the reduction of CNS side effects of this molecule [1]. The mode and location of action may be manyfold. Galcanezumab is thought to block neuronal signal transmission within the trigeminal pain system. This includes the trigeminal ganglion as well as axon-to-axon signaling transmission within the trigeminal Aδ-fibers at the nodes of Ranvier [5, 6]. Since Galcanezumab is not selective for any kind of CGRP within any specific tissue, this substance blocks CGRP throughout the entire body [5].

7.3 Preclinical Trials

The preclinical testing of Galcanezumab was performed in a human forearm blood flow model in which the property of the antibody to inhibit capsaicin-induced dermal blood flow (DBF) was studied [7]. Galcanezumab doses of 1, 5, 25, 75, 200, and 600 mg (n = 7/dose) or placebo (n = 2/dose) were injected s.c. in six groups of volunteers (n = 9/group) and measures were performed 48–56 h after the last subcutaneous injection [7]. A multiple dosing group was also included [7]. The pharmacokinetics of Galcanezumab was linear in this study with increasing doses leading to increasing serum concentrations [7]. In addition, Galcanezumab inhibited capsaicin-induced DBF dose-dependently, with an effect of a dose as low as 5 mg [7]. Doses of 75–600 mg had the most pronounced inhibitory effect on capsaicin-induced DBF by day 3 until day 42 [7].

In summary, Galcanezumab demonstrated inhibition of capsaicin-induced DBF in a concentration–response relationship. Based on these studies, doses of 150 and 300 mg Galcanezumab were studied in the phase II clinical trial program for migraine prophylaxis.

7.4 Phase II Clinical Trial Program for the Prevention of Migraine

Galcanezumab (LY2951742) was studied in the prevention of episodic migraine in a randomized, placebo-controlled, double-blind clinical trial in adults with 4–14 monthly migraine days (MMD) [8]. The drug was administered twice a month in a dose of each 150 mg s.c. in a 1.5 mL solution or an identical volume of placebo over a trial period of 3 months [8]. The trial did not allow any stable preventive

co-medication and excluded patients with more than two prior preventive treatment failures. Patients with medication overuse could also not enroll into the trial, but the intake of up to four barbiturates per month was allowed in the baseline and the entire trial period. The use of abortive antimigraine medication was permitted in all study phases [8]. The primary endpoint of this trial was the mean change of MMD during weeks 9–12 versus baseline in the LY2951742 group compared to placebo. Safety assessment included the adverse events in the trial period and a 12-week follow-up period [8]. Secondary endpoints of this trial were the mean change of headache days (HD) per 28-day period as well as the responder rates [8]. The trial was conducted in the United States, with 82% of female participants. Patients were mostly Caucasian (71%) with a high body mass index of 29 kg/m^2 [8]. Electronic diaries revealed 6.8 MMD in the LY2951742 group and 7.0 MMD in the placebo group during baseline. Active drug led to a reduction of −3.7 MMD within the first 4 weeks while placebo reduced MMD by 2.3 days [8]. At the time of the primary endpoint (weeks 9–12), LY2951742 was clearly superior to placebo with a reduction of 1.2 MMD greater than placebo (LY2951742 –4.2 MMD vs. placebo −3.0 MMD) [8]. The 50% responder rate was 72% for the active compound, while 45% of patients on placebo reached a 50% or greater reduction of MMD [8]. This unusually high placebo response in a migraine prevention trial may be related to the twice-monthly dosing regimen.

The reduction of monthly HD was in line with the reduction of MMD with −3.7 days (placebo) vs. −4.9 days (LY2951742) [8]. Interestingly, about 32% of patients had a complete response, meaning no migraine days in the last 4 weeks of the double-blind trial period in the LY2951742 arm (vs. 17% of patients on placebo) [8].

More than 70% of patients in both groups reported adverse events [8]. Upper respiratory tract infections, pain at the injection site, and erythema were most frequently mentioned in both groups. Five patients in the LY2951742 arm had hypertension but none in the placebo group. The finding of hypertension was not reproduced in phase III trials, maybe due to the change of the bimonthly dose regimen in subsequent trials [9–11]. Treatment-related serious adverse events were not reported in this study.

LY2951742 was effective in this phase II trial and further assessed in the prevention of migraine [12]. In all future trials, LY2951742 was named Galcanezumab due to the name assignment of the US Adopted Names Program.

7.5 Phase III Registration Trials for the Prevention of Episodic Migraine

In this trial program, two Galcanezumab doses were assessed. One scheme studied a monthly 240 mg s.c. dose, while the other had a loading dose of 240 mg followed by monthly 120 mg s.c. injections of Galcanezumab [9–11]. The latter is the approved dose regimen of Galcanezumab for clinical use across the globe.

The clinical trial program for the prevention of episodic migraine with Galcanezumab was entitled EVOLVE (Evaluation of Galcanezumab for the Prevention of Episodic Migraine). EVOLVE-1 was performed in the United States and Canada, while EVOLVE-2 also included patients from Europe and other regions [9, 10]. Both trials were identically designed. The program consisted of a 4-week baseline phase, followed by a 6-month randomized, placebo-controlled, double-blind treatment period in which the patients received Galcanezumab in the doses mentioned above or identical volume of placebo [9, 10]. The double-blind study phase was followed by a 4-month follow-up period. In addition, a long-term open-label safety trial was performed in all phase III trials [9–11].

The primary endpoint of both EVOLVE studies was the mean change of MMD from baseline during the entire double-blind treatment period of Galcanezumab in two different doses in comparison to placebo [9, 10]. This scheme is unique as it considers the efficacy of the substance of interest from day 1 of the first injection over the entire 6-month double-blind trial period. These studies included patients with 4–14 MMD [9, 10]. Acute abortive medication was allowed [9, 10]. Patients who failed three or more classes of preventative were excluded from the trials. The predefined secondary endpoints were the 50%, 75%, and 100% reduction rates from baseline in the number of MMD over months 1–6, the overall mean change from baseline in the number of monthly days with acute medication use, and the impact of migraine on daily activities as an indirect measure of quality of life (QoL) [9, 10].

Patients in the EVOLVE trials were on average 41 years old and mostly female (84–85%) with 9.1/9.2 MMD during baseline, which translates into an attack frequency of 5.5–5.8/month [9, 10]. On average, patients documented 7.4–7.6 days with acute medication intake/month [9, 10]. Sixty percent of the study participants had prior preventive migraine treatment attempts.

The primary endpoint was met in both EVOLVE trials [9, 10]. A dose of 120 mg Galcanezumab reduced MMD by −4.3 to −4.7 on average with a difference between 1.9 and 2.0 MMD to placebo. Already by month 1, Galcanezumab was superior to placebo. The 50%, 75%, and 100% rates were also in favor of the active substance [9, 10]. For example: In EVOLVE-1 62% of patients reached a 50% or greater reduction in MMD, 39% a greater than 75% reduction of MMD, and 16% of patients reached migraine freedom on an average month during the trial [9]. In line with the reduction of MMD, Galcanezumab led to a reduction of days with acute medication use by almost 4 compared to a reduction of 2.2 days in patients on placebo.

Subgroup analyses revealed that already in week 1 after the injection of the loading dose the number of patients with migraine headache is reduced with Galcanezumab [13]. Already on the first day after injection, fewer patients with active drug experience migraine headache than patients on placebo, and this pattern continues over the entire first treatment week [13]. It is also very interesting that despite the natural degradation of Galcanezumab there is no wearing-off phenomenon in the last week of the 4-week period between injections [9, 10]. The change of MHD after injections did not differ between weeks in a given treatment cycle with the exception of week 4 in the period following the loading dose (vs. week 1)

[14]. Of note, active study drug led to significantly greater percentages of patients achieving a >75% reduction at each month, starting at month 1, with 26% of patients with a greater than 75% reduction, which increases to 44% by month 6. Thirty-nine percent (39%) of patients had at least 1-month migraine freedom in both studies and 11% have at least 3 months of migraine freedom with Galcanezumab [15].

7.6 Phase III Registration Trials for the Prevention of Chronic Migraine

In the chronic migraine trial called REGAIN, participants were treated over a 3-month period with Galcanezumab or placebo [11]. The same endpoints as in the EM program have been used. The participants in this study had on average 19.4 MMD during the 4-week baseline. Patients took acute medication 15.5 days per month and 78% had taken prior preventive treatment [11]. Sixty-five percent (65%) of patients with migraine failed >2 preventative prior to inclusion in this trial [11].

The primary endpoint, which was the reduction of MMD over months 1–3, was reached [11]. Patients on Galcanezumab had a reduction of −4.8 MMD versus −2.7 MMD on placebo. Similar to EVOLVE, onset of efficacy could be demonstrated within the first month of treatment in a trial with over 800 patients. Patients on Galcanezumab had almost 5 days less/month with acute medication use in weeks 9–12 and ~28% of subjects had a 50% reduction in MMD [11].

Safety and tolerability of Galcanezumab were not different between trials [9, 10]. The safety analysis led to the finding that serious adverse events were also not different between all groups (Table 7.1). Less than 1.5% of patients reported serious adverse events. Treatment-emergent adverse events were reported by 62.6% of patients on 120 mg Galcanezumab, 64.7% on 240 mg Galcanezumab, and 57% of patients on placebo. Less than 2.5% of patients on active drug discontinued due to treatment-related events. Over 80% of patients (82–88%) completed the EVOLVE trials and 95% completed the chronic migraine study with a shorter double-blind treatment duration of 3 months [9, 10]. It is important that a great majority of patients (>80%) reported experiencing less impact from side effects compared to their previous treatments over the 12-month treatment period of a long-term, open-label safety study ($n = 270$) [16].

Current treatment guidelines recommend stopping monoclonal antibody therapy after 9–12 months. This advice is based on the historic approach with unspecific oral migraine preventatives with numerous side effects. In a small real-world study in patients with chronic migraine receiving monthly Galcanezumab or Erenumab for 9 months, patients continued to benefit after treatment termination for about 16 weeks after the last drug injection [17]. However, headache and migraine frequency were slowly increasing over time but had not reached baseline levels at the end of the observation period [17]. A similar finding was observed in a larger cohort of patients treated with Galcanezumab [18].

Table 7.1 List of adverse events in Galcanezumab clinical trials

System organ class	Very common (≥1/10)	Common (≥1/100 to <1/10)	Uncommon (≥1/1000 to <1/100)	Rare (≥1/10,000 to <1/1000)
Immune system disorders				Anaphylaxis Angioedema
Ear and labyrinth system		Vertigo (0.7%–1.2%)		
Gastrointestinal system		Constipation (1.0%–1.5%)		
Skin and subcutaneous tissue		Pruritus (0.7%–1.2%) Rash	Urticaria (0.3%–0.1%)	
General disorders and administration site conditions	Injection site pain (10.1%–11.6%) Injection site reactions (9.9%–14.5%)			

7.7 CONQUER—A Phase IIIb Clinical Trial in a Difficult-to-Treat Cohort

The CONQUER trial completed a series of studies of the s.c. administered mAbs in a more difficult to treat patient population [19]. CONQUER enrolled 462 patients with episodic and chronic migraine, who had not improved to previous treatments with two to four different prophylactic medication categories in the maximal tolerated dose for 8 weeks or had to stop such medications due to side effects within the past 10 years [19]. The trial also included patients up to an age limit of 75 years and did not consider medications for inclusion and exclusion, which could not have been prescribed previously due to contraindications [19].

The difference to the LIBERTY trial with Erenumab relates to the population [20]. LIBERTY studied patients with episodic migraine and failures to specific medications; only the two β-blockers metoprolol and propranolol formed a group [20]. The trial had a rather complex design, in which every patient must have had a failure to a first-line migraine preventative, i.e., β-blocker or flunarizine or topiramate or amitriptyline [20]. This was not the case in the design of the FOCUS and CONQUER trials for Fremanezumab and Galcanezumab [19, 21]. The strength of these trials relates to the population studied as this consisted of an episodic and chronic population [19, 21]. FOCUS grouped treatment failures according to medication classes, while CONQUER had grouped medications into different categories [19, 21].

In CONQUER, patients from 18 to 75 years of age with an average of 13.4/13.0 MMD during baseline received a loading dose of 240 mg Galcanezumab s.c. or placebo s.c. followed by monthly s.c. injections of 120 mg of active drug or placebo,

respectively [19]. Over the entire 12 weeks observation period, the reduction of MMD was 3.1 days greater with Galcanezumab than with placebo [19]. Galcanezumab led to a reduction of −4.1 MMD while the response to placebo was −1.0 [19]. Already in the first month, Galcanezumab showed efficacy superior placebo [19].

The study also met the key secondary endpoint, which assessed the change of MMD of Galcanezumab versus placebo in the episodic migraine subpopulation [19]. These patients with episodic migraine had 9.34 MMD during baseline, with no difference between groups [19]. In this subgroup, which resembled ~60% of patients in the trial, Galcanezumab led to a reduction of −2.88 MMD, which was superior to placebo [19]. The frequency of MMD in the chronic migraine subpopulation was reduced by 5.9 days with Galcanezumab versus −2.21 days with placebo [19]. The prespecified gated secondary endpoints such as the ≥50%, ≥75%, or 100% responder rates and the improvement of functioning as measured by the Migraine Specific Quality of life questionnaire—Role Function Restrictive domain (MSQ v2.1 ePRO RFR) revealed a positive effect of the study drug in the entire population and the subgroup with episodic migraine [19]. The >50% response rate was achieved by 24% more patients in the Galcanezumab study group (see Table 7.2) [19].

After a 3-month double-blind treatment period, all patients received Galcanezumab for another 3 months in an open-label fashion [19]. Patients new on Galcanezumab had impressive improvement in the first treatment month while patients continuing with Galcanezumab continued to improve. The reduction of migraine days was not different between groups already at the end of month 4 (month 1 of the OLE) with a reduction of MMD between −4.89 and −5.6 [19]. The analysis revealed no new safety findings. Only five patients discontinued treatment due to adverse events [19].

Across all trials, antidrug antibodies (ADA) were detected in 2.6–12.4% of patients on Galcanezumab with a peak occurrence in months 3–6 [22]. Surprisingly, some patients have ADA at baseline. Most patients with ADA have neutralizing ADA, but without a clear effect on the efficacy of Galcanezumab [22].

Table 7.2 Secondary endpoints of the CONQUER trial [19]

	Total		
Endpoint	PBO *N* = 228	GMB 120 mg *N* = 230	*P* value
≥50% response rate	13.3%	37.7%	<0.0001
≥75% response rate	3.3%	14.5%	<0.0001
100% response rate	0.0%	4.9%	<0.0001
MSQ-RFR	10.7	23.2	<0.0001

GMB Galcanezumab, *MSQ-RFR* Migraine-Specific Quality of Life Questionnaire Role Function–Restrictive, *PBO* Placebo

7.8 Functional Improvement with Galcanezumab

For patients, quality of life and functional improvement are both important topics. Several different quality of life questionnaires have been established over the years. Among these, the Migraine Disability Assessment Test (MIDAS) and the Migraine-Specific Quality-of-Life Questionnaire (MSQ) were used to evaluate the efficacy of Galcanezumab in patients with episodic and chronic migraine, and in more refractory patients (CONQUER) on the improvement of QoL [23, 24].

In order to provide an overview, we will illustrate Qol data from several studies.

For example, we would like to explain the MIDAS questionnaire [23]. The MIDAS is a five-item self-administered questionnaire that sums the number of productive days lost over the past 3 months in the workplace and assesses disability in family, social, and leisure activities at home (e.g., How many days in the last 3 months was the patient at least 50% disabled at work, home, school, or recreational activities due to migraine?). In addition to a total score, there are subdomains of absenteeism (missed days due to a headache from paid work, housework, and nonwork activities) and presenteeism (days at paid work or housework where productivity was reduced by at least half). MIDAS scores are interpreted as Grade I = 0–5 (minimal or infrequent disability), Grade II = 6–10 (mild or infrequent disability), Grade III = 11–20 (moderate disability), and Grade IV = 21 and over (severe disability). Although no minimally important difference (MID) has been established for MIDAS, a preliminary analysis based on an anchor of 25% change in monthly headache days estimated that an increase or decrease of 5 days of migraine-related disability per 3 months represents meaningful within patient change.

Across all Galcanezumab trials, the MIDAS scores have improved with Galcanezumab greater than with placebo [9–11, 25]. In the CONQUER trial patients in both groups had a median MIDAS score at baseline of ~50 points (50.96 in the placebo group and 50.90 in the Galcanezumab group), indicating severe disability on inclusion. After 3 months of double-blind treatment, the MIDAS score improved by greater than 20 points in the Galcanezumab group, while patients on placebo showed less than five points improvement. This indicates that patients with Galcanezumab are less disabled after 3 months Galcanezumab than during baseline and less disabled than patients on placebo. In line with these findings, all three MSQ domains (Role Function restrictive, Role function preventive, and Emotional function) have significantly greater improved with Galcanezumab than with placebo. Improvement could already be detected by month 1. Another score assessing work productivity (Work Productivity and Activity Impairment Questionnaire, WPAI) showed that the overall work impairment can be improved with Galcanezumab to a larger extend than with placebo [26]. Finally, days missed of work or school were reduced in the EVOLVE study program with Galcanezumab [25]. In EVOLVE-2, a reduction of 1.7 days from baseline (2.8 days) was observed by month 6 versus an improvement of 0.8 days with placebo [25].

In summary, across several scoring instruments, Galcanezumab showed efficacy in improving quality of life in patients with migraine.

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Chapter 8
Eptinezumab

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8.1 Introduction

Eptinezumab-jjmr (ALD403, VYEPTI™; Lundbeck A/S, Copenhagen, Denmark) is a genetically engineered, desialylated, humanized gamma immunoglobulin 1 kappa ($IgG_{1\kappa}$) targeting both α- and β-calcitonin-gene-related peptide (CGRP) ligands with a picomolar affinity. It is the first and only intravenous formulation in its class. It was initially developed by Alder Biopharmaceuticals, Inc., which was later acquired by Lundbeck A/S in October 2019. The US Food and Drug Administration (FDA) approved its use in February 2020 for migraine prevention in adults. The recommended dosage is 100 mg as an intravenous infusion every 3 months, where some patients may benefit from a dosage of 300 mg.

8.2 Development

Eptinezumab was engineered via unique technologies to achieve high target specificity, reduced immunogenicity, and efficient manufacturing. The high-affinity

Disclosure: Dr. Yuan received honoraria from Supernus Pharmaceuticals, Inc. Dr. Silberstein receives, or has received, honoraria from AbbVie.; Amgen; Aeon BioPharma; Axsome Therapeutics; Biohaven Pharmaceuticals; Cefaly; Curelator, Inc.; Epalex; GlaxoSmithKline Consumer Health Holdings, LLC.; electroCore Medical, LLC; Impel NeuroPharma, Inc.; Lilly USA, LLC; Medscape, LLC; Lundbeck; Nocera; Novartis, Inc.; Revance: Salvia, Bioelectronics; Satsuma Pharmaceuticals; Supernus Pharmaceuticals, Inc.; Teva Pharmaceuticals; Theranica; Thermaquil; and Trillen Medical Inc. No funding was received for this manuscript.

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_8

monoclonal antibody was generated initially from in vitro biopanning via B cell culture, enzyme-linked immunosorbent assay (ELISA), and reverse transcription polymerase chain reaction (RT-PCR). Briefly, B cells from rabbits were immunized with human CGRP, screened for antibody CGRP binding by ELISA, and used for antibody production. Once binding specificity was confirmed, genes from these antibodies' single heavy- and light-chain variable regions were identified by RT-PCR and expressed as recombinant antibodies then screened for optimal CGRP affinity. Subsequently, these antibodies underwent humanization to retain antigenic specificity but reduce immunogenicity. Humanization entails grafting the antigen-binding regions, so-called the complementarity-determining regions (CDRs), to the human variable region framework sequences. To optimize its CGRP functional blocking property, as measured by in vitro cAMP inhibition potency, 13 amino acids between the light- and heavy-chain human framework sequences were reverted to the rabbit sequence [1]. To further reduce immunogenicity, some modifications (e.g., desialyzation) were made in the fragment crystallizable (Fc) region. Since activation of Fc-mediated immune response depends heavily on the N-linked glycan at amino acid 297 on IgG, canonical asparagine 297 in the heavy chain was specifically mutated to alanine (N297 mutation) to avoid interactions with Fcγ receptor and complement protein [1]. To improve manufacturing efficiency, yeast was used instead of mammalian cells. Through collaboration with Keck Graduate Institute of Applied Life Sciences, a novel technology (MabExpress), was used to create recombinant polypeptides generated from *Pichia pastoris* (now a standard tool for the production of recombinant protein in molecular biology) [2]. The benefits of *P. pastoris* include appropriate folding and secretion of recombinant proteins to the external environment of the cell as well as simple purification of recombinant protein in the *P. pastoris* expression system due to its limited production of endogenous secretory proteins. Compared with mammalian cells, *P. pastoris* offers additional advantages, such as rapid fermentation cycle time, shorter cell-line selection time, greater scale of production, and no viral clearance requirement.

Among several final antibody candidates, ALD403 was chosen for its optimal balance between CGRP affinity/specificity, immunotolerance, and manufacturability. Intravenous formulation was chosen over subcutaneous formulation for its better pharmacokinetic profile. In vitro studies showed that ALD403 binds to both forms (α and β) of human CGRP but not to other neuropeptides (e.g., amylin, calcitonin, adrenomedullin, intermedin); it inhibits CGRP binding with an IC_{50} of 47 pM. By eliminating the N-linked glycosylation on the canonical N297 residue, ALD403 did not induce antibody-dependent cellular cytotoxicity, stimulate complement-dependent cytotoxicity, nor interact stably with any of the Fcγ receptor mediating these functions. Clinical studies further confirmed its reduced immunoclearance, effective CGRP blocking, and quicker onset of action.

8.3 Administration

Eptinezumab is formulated in a single-dose vial (1 mL) with no preservative. Each vial contains excipients of histidine, histidine hydrochloride, polysorbate 80, sorbitol, and water with a pH of 5.8. Upon dilution to 100 mL normal saline, Eptinezumab is infused (not push or bolus) over approximately 30 minutes through 0.2 or 0.22-μm sterile filter. The diluted solution should be infused within 8 h. After the infusion is complete, the line is flushed with 20 mL saline. The recommended dose is 100 mg every 3 months, while some patients may benefit from 300 mg. Those with hypersensitivity reactions to eptinezumab or its excipients should consider discontinuing infusion and institute appropriate therapy.

At the moment, there is no sufficient safety data of its use in pregnant/nursing women or patients <18 or >65 years old. Since CGRP has roles in tissue healing, hematopoiesis, neuro-immune axis, placental decidualization, and cardiac protection, the use of CGRP functional blockade in susceptible populations (e.g., sepsis, stroke, ischemic heart, pregnancy) should be done with caution. Postmarketing pregnancy registry and pregnancy outcome studies, as well as pediatric studies, are required by the FDA.

8.4 Pharmacokinetics

The pharmacokinetics of eptinezumab shares the general property of antibodies but has some unique features. IgG pharmacokinetics vary between individuals by body size, age, sex, and many other factors [3]. In general, eptinezumab's systemic distribution depends on passive extravasation and convection through blood vessels into tissues. Upon administration, IgGs are generally distributed in organs with leaky vasculatures, such as skin, liver, and spleen. In the central nervous system, IgGs do not cross the blood–brain barrier. In contrast, dura and sensory ganglia are highly permeable [4] and are eptinezumab's possible sites of action. Inside trigeminal ganglia, labeled fremanezumabs were found surrounding individual neurons and satellite glial cells; [5] eptinezumab likely exhibits a similar distribution. Unlike small molecules, IgGs are not metabolized by the liver nor filtered by the kidneys; thus there is no need for dose adjustment in patients with hepatic or renal dysfunction. Instead, IgGs are metabolized inside endothelial cells upon nonspecific pinocytosis. IgG catabolism is estimated to be 33%, 24%, 16%, and 12% from skin, muscle, liver, and gut, respectively [6]. Its extended circulatory half-life is primarily mediated by the neonatal Fc receptors. Binding to the neonatal Fc receptors in the endosomal compartment allows IgGs to escape intracellular degradation. However, significant variation exists in the pharmacokinetics between individuals with the same IgG or even the same individual with time [7].

Eptinezumab, following intravenous administration, reaches maximal serum concentration at the end of infusion with 100% bioavailability and a half-life of 27 days [8]. Compared to other migraine preventive monoclonal antibodies, eptinezumab has a shorter time to maximal concentration (C_{MAX}) and greater exposure (assessed by C_{MAX} and area under curve) upon administration [9]. This may explain the rapid treatment effect for eptinezumab as early as day 1 [10]. Eptinezumab most likely does not interact with small molecules as coadministration of single-dose eptinezumab 300 mg with single-dose subcutaneous sumatriptan 6 mg did not affect the pharmacokinetics of either drug [11]. However, it is worth noting that the interactions of antibodies and small molecules are complex and not fully understood.

8.5 Pharmacodynamics and Mechanism of Action

Eptinezumab potently (K_D < 20 pM) and selectively binds to both α and β forms of human CGRP. It has extensive contact between all six CDRs and α-CGRP with a high ligand-binding surface area (797 Angstrom) [12]. In binding analyses using surface plasmon resonance, galcanezumab and eptinezumab engaged the CGRP ligand quicker (higher K_a) than fremanezumab, whereas eptinezumab and fremanezumab dissociated much slower (smaller K_d) than galcanezumab [13]. Eptinezumab and fremanezumab both essentially bound to CGRP with undetectable dissociation over 24 h. Theoretically, this behavior eliminates background concern of these antibodies "leaking" CGRP. However, the IgG binding is a thermodynamic process that favors association over dissociation rather than a 1-to-1 fixed binding [14, 15]. At any given time, there will always be both free IgGs and their ligands. This may explain why CGRP's functional blockade reaches a plateau despite higher IgG concentration.

Eptinezumab behaves similarly to other CGRP functional blocking monoclonal antibodies by modulating CGRP-related nociceptive signaling, neurogenic inflammation, and pain sensitization in migraine. A detailed discussion on its mechanism of action is beyond the scope of this article. However, there are several pertinent animal studies of eptinezumab to date. Specifically, pretreatment of eptinezumab inhibited capsaicin-driven blood flow in a dose-dependent fashion [1]. Eptinezumab alleviated and prevented the cutaneous mechanical hypersensitivity induced by nitroglycerin in rats [16]. When administered intracerebroventricularly in mice, eptinezumab failed to change the mechanical sensitivity threshold suggesting a lack of central action [17]. Eptinezumab also attenuated the peripheral CGRP-induced light aversion behaviors and diarrhea in mice [18, 19].

8.6 Eptinezumab Clinical Trials

There are seven eptinezumab clinical trials registered to date (Table 8.1). In its phase 1 study (NCT01579383), ALD403 ranging from 1 to 1000 mg versus placebo was administered intravenously or subcutaneously to healthy volunteers. The

Table 8.1 Summary of eptinezumab-related clinical trials

Trial information	Dose (mg)	Primary endpoint(s)
NCT01579383 [20] Phase 1, HV	1–1000 vs. Plc	Safety and tolerability.
NCT01772524 [21] Phase 1b, EM, $n = 163$	1000 vs. Plc	Safety profile in 24 weeks. (Plc-adjusted MMD change: −1.0)[a]
NCT02275117 [22] Phase 2b, CM, $n = 616$	300, 100, 30, 10 vs. Plc	≥75% responder rates: 33.3%*, 31.4%*, 28.2%, 26.8%, vs. 20.7% (Plc-adjusted MMD change: −2.7*, −2.1*, −2.4*, −1.2)[a]
NCT02559895 [24] Phase 3, EM, $n = 888$	300, 100, 30 vs. Plc	Plc-adjusted MMD change: −1.1*, −0.7*, −0.8
NCT02974153 [25] Phase 3, CM, $n = 1072$	300, 100 vs. Plc	Plc-adjusted MMD change: −2.6*, −2.0*
NCT02985398 [29] Phase 3, CM, n=128[b]	300	TEAE or other safety event at 104 weeks.
NCT04152083 [32] Phase 3, EM, $n = 480$	100 vs. Plc	Time to headache pain freedom and absence of MBS

All studies were evaluated over 12-week unless otherwise specified. HV: healthy volunteer. *EM* Episodic migraine, *CM* Chronic migraine, *Plc* Placebo, *MMD* Mean monthly migraine days, *TEAE* Treatment-emergent adverse event, *MBS* Most bothersome symptom

[a]Secondary endpoint

[b]Open label

*Statistically significant

primary endpoint was safety and tolerability. In the published pharmacokinetics and pharmacodynamics data, [20] the half-life was approximately 26 days and the bioavailability for subcutaneously administered ALD403 was 70%. Subjects receiving ALD403 had dose-dependent reductions in mean % baseline capsaicin/vehicle dermal perfusion ratios relative to placebo, such reduction persisted for 12 weeks after single administration.

In its first efficacy study (NCT01772524, phase 1b), 174 subjects (age 18–55) with episodic migraine (EM; 5–14 migraine days in 28 days) were randomized, and 163 subjects were allocated for a single dose of ALD403 (1000 mg in 1-h infusion) or placebo [21]. Per clinicaltrial.gov, the study's primary outcome measure was safety, and the secondary outcome measures were pharmacokinetic parameters and clinical efficacy. Two patients in the placebo group and five in the ALD403 group were lost to follow-up during the 12-week study. No patient withdrew because of the absence of efficacy or adverse events (AEs); 57% and 52% of subjects in the treatment and placebo group, respectively, experienced treatment-emergent adverse events (TEAEs) with the most common being upper respiratory tract infection, urinary tract infection, fatigue, back pain, nausea and vomiting, and arthralgia. No

infusion reactions were reported. Six serious adverse events (SAEs) were reported by three patients; all of these events were deemed unrelated to ALD403. There were no clinically significant differences in vital signs, 12-lead ECGs, or laboratory safety data between patients treated with ALD403 or placebo. In their efficacy analysis, at 9–12 weeks, the mean monthly migraine days decreased by 5.6 ± 4.0 (ALD403) and 4.6 ± 3.5 (placebo) days with no statistical difference but was statistically (one-sided test) different at weeks 5–8 (5.6 ± 3.0 vs. 4.6 ± 3.6). The 50%, 75%, and 100% reduction in migraine days were numerically higher in the treatment groups at all timepoints (no statistical analysis reported). The mean apparent terminal elimination half-life was 27.9 (range 19.9–46.5) days. The mean total plasma clearance was 0.125 ± 0.038 L/day, and the mean terminal phase distribution volume was 4.98 ± 1.83 L.

In a multicenter exploratory phase 2b study (NCT02275117) for the prevention of chronic migraine (CM), 665 subjects were randomized and 616 subjects received a single dose (100 mL in 1-h infusion) of eptinezumab 300 mg, 100 mg, 30 mg, 10 mg, or placebo [22]. The primary endpoint was monthly ≥75% responder rate over weeks 1–12. One study site (n = 28) was excluded due to multiple protocol violations. Only 25 subjects withdrew from the study with nine withdrawn due to lack of efficacy within 12 weeks. Sixty-five percent of subjects received no concomitant preventive medication; the most common concomitant preventive was topiramate (n = 95; 15.4%). In the primary efficacy analysis, the average monthly ≥75% migraine responder rates were 33.3%, 31.4%, 28.2%, and 26.8% for eptinezumab 300, 100, 30, and 10 mg, respectively, compared with 20.7% for placebo (p = 0.033, 0.072, 0.201, and 0.294 vs. placebo). Mean monthly migraine days decreased from 16.5, 16.9, 16.2, and 16.4 versus 16.4 at baseline to 8.3, 9.3, 8.3, and 9.7 versus 10.9 for eptinezumab 300, 100, 30, and 10 mg versus placebo, respectively (p = 0.003, 0.018, 0.005, 0.180). HIT-6 showed changes in baseline scores of −10.0, −6.9, −6.5, and − 6.5 for the 300, 100, 30, and 10 mg groups, respectively, compared with −5.8 for the placebo group. Other secondary endpoints, such as ≥50% responder rates, monthly headache days, and migraine frequencies, also favored eptinezumab 300 and 100 mg over placebo. Percentages of patients who experienced a migraine on day 1 post-infusion of the 300 and 100 mg doses were 26.3% and 29.3% versus 48.7% for placebo. In a post hoc analysis, 299 blood samples were analyzed using microarray-based genome analysis on eight genes centrally related to CGRP biology. No statistically significant relationship was identified between any level of clinical response and genotype/copy number status [23]. TEAEs were reported in 345 subjects with the most common being upper respiratory tract infection and dizziness. Thirteen reported 16 SAEs; none were considered related to eptinezumab. Ten had an interruption of infusion due to hypersensitivity, all of which were mild to moderate in intensity and resolved within 24 h after symptomatic treatment. Antidrug antibody (ADA) response was found maximal at 24 weeks with an incidence around 18–19% (5.8% neutralizing) in subjects who received 100 or 300 mg of eptinezumab although no apparent impact on efficacy was noticed in 12 weeks.

The efficacy and safety of eptinezumab for migraine prevention were evaluated in three phase 3 clinical trials (two controlled and one open-label). In the PROMISE-1 (PRevention Of Migraine via Intravenous eptinezumab Safety and Efficacy 1) study, 898 subjects (age 18–75) with EM (≥4 migraine days and ≤14 headache days in 28-day screening period) were randomized and 888 subjects received eptinezumab (100 mL in 1-h infusion) 300 mg, 100 mg, 30 mg, or placebo [24]. No regular use (>7 days) of preventive medication, botulinum toxin use within 4 months, >4 days/month of opioid/barbiturate use, or >14 days/month of acute medication use was allowed. The primary endpoint was the change from baseline in monthly migraine days in 28-day interval averaged across weeks 1–12. Subjects received up to four treatments of quarterly eptinezumab or placebo for long-term safety up to 56 weeks; 94.0% completed the 12-week efficacy period, and 23.9% discontinued the safety study early (15% withdrawal by patient, 7.5% loss to follow-up). In the primary efficacy analysis, mean monthly migraine days were 8.6, 8.7, 8.7, versus 8.4 at baseline to 4.3, 4.7, 4.6 versus 5.4 for eptinezumab 300 mg, 100 mg, 30 mg versus placebo ($p = 0.0001$, 0.0182, 0.0046; the last being unadjusted for repeated measurement). Key secondary efficacy endpoints, such as ≥75% and ≥50% migraine responder rates, favored eptinezumab statistically. Almost 60% experienced at least one TEAE, with the most common (≥2%) being upper respiratory tract infection, nasopharyngitis, and sinusitis; 2.8% had SAEs and 3.3% led to withdrawal. The incidence of ADA was maximal at 24 weeks (15.3%), where 44.8% of the 87 ADA-positive subjects were having neutralizing antibodies. ADA exhibited no impact on the efficacy and safety profile of eptinezumab. Overall, eptinezumab 100 and 300 mg were effective and well-tolerated in patients with EM.

In the PROMISE-2 study, 1121 subjects (age 18–65) with CM (15–26 headache days and ≥8 migraine days in 28-day screening period) were randomized, and 1072 received eptinezumab (100 mL in 30-minute infusion) 300 mg, 100 mg, or placebo quarterly for 6 months. Stable acute/preventive medications were allowed but botulinum toxin use within 4 months or barbiturates/opioids >4 days/month were not [25]. The primary endpoint was the change from baseline in monthly migraine days (weeks 1–12); 93.6% remained in the study until week 12. In the primary efficacy analysis, mean monthly migraine days were 16.1, 16.1 versus 16.2 at baseline to 7.9, 8.5, versus 10.5 during week 1–12 for eptinezumab 300 mg, 100 mg, placebo, respectively ($p < 0.0001$ in both doses). Key secondary efficacy endpoints, such as ≥75% and ≥50% migraine responder rates, also favored eptinezumab statistically. Compared to placebo, subjects were more likely to achieve ≥75% and ≥50% response for eptinezumab 300 mg (odds ratio 2.8 [95%CI 1.9–4.0] and 2.4 [95%CI 1.8–3.3]) and 100 mg (odds ratio 2.0 [95%CI 1.4–3.0] and 2.1 [95%CI 1.6–2.8]). The reduction in migraine that occurred on day 1 was maintained throughout the study. Subjects demonstrated significant improvement on HIT-6 with a difference of −2.9 (95%CI −3.9 to −1.8) for 300 mg and −1.7 (95%CI −2.8 to −0.7) for 100 mg, as well as acute medication use reduction of −1.4 (95%CI −1.9 to −0.9) for 300 mg and −1.2 (95%CI −1.7 to −0.6) for 100 mg. In a post hoc subgroup analysis, subjects with medication overuse (40.2%) responded to eptinezumab over week 1–12 with monthly migraine day reduction of −8.5, −8.2 versus −5.2 for 300 mg, 100 mg

versus placebo [26]. Overall, 47.4% reported TEAEs; most common (≥2%) were nasopharyngitis, upper respiratory tract infection, sinusitis, urinary tract infection, migraine, nausea, and fatigue. Ten (<0.1%) subjects experienced SAEs, and six subjects withdrawn due to hypersensitivity. The incidence of ADA was maximal at 24 weeks (17.1%), where 21.4% of 112 ADA were neutralizing. ADA exhibited no impact on the efficacy and safety profile of eptinezumab. Overall, eptinezumab 100 and 300 mg were effective and well-tolerated in patients with CM.

Eptinezumab was generally well tolerated in patients with EM or CM. According to a pooled data from PROMISE 1 and 2 studies, over 6 months of treatment eptinezumab resulted in a greater number of study months with ≥75% and ≥50% migraine response than placebo. Early migraine response was associated with a greater likelihood of sustained response across treatment arms [27]. The most common AEs (incidence ≥2% and ≥2% more than placebo) in eptinezumab 300 mg ($n = 574$) and 100 mg ($n = 579$) versus placebo ($n = 588$) recipients were nasopharyngitis (8%, 6% vs. 6%) and hypersensitivity (2%, 1% vs. 0%). Adverse reactions resulted in treatment discontinuation in 1.9% of subjects [28].

In an ongoing open-label Phase 3 TRial to EValuate the Safety of Eptinezumab Administered IntravenousLy in Patients with CM (PREVAIL), 128 subjects (age 18–65) completed the primary treatment phase (four infusions of eptinezumab 300 mg, 12 weeks apart) and 87.5% received the 4th dose. TEAEs were reported for 64.8% of patients, with the most common (≥5%) being nasopharyngitis (13.3%), upper respiratory tract infection (7.0%), sinusitis (6.3%), and influenza (5.5%); 7.8% had SAEs [29]. There were substantial reductions in the Migraine Disability Assessment (MIDAS) score (mean change of −36.3, −40.3, −41.2, −40.2 at month 3, 6, 9, 12) and Headache Impact Test (HIT-6) score (mean change of −7.9, −9.1, −9.3, −8.3 at month 3, 6, 9, 12). The magnitude of the effect was maintained through month 12 [30, 31].

At the time of writing, a multicenter clinical trial (NCT04152083, RELIEF) investigating eptinezumab 100 mg IV in EM subjects experiencing acute migraine attacks was completed. Treatment was given within 1–6 h of headache onset. The co-primary endpoints are the time to headache pain freedom and the time to the absence of most bothersome symptom (MBS). Pain freedom and absence of MBS at 2 h post start of infusion are key secondary endpoints. The study met statistical significance on the co-primary endpoints and key secondary endpoints, but the full data remain to be published [32].

8.7 Conclusion

Eptinezumab (formerly ALD403), a humanized therapeutic IgG_1 monoclonal antibody targeting CGRP and manufactured using yeast (*P. pastoris*), is effective in the treatment of migraine. It reduces monthly migraine days and acute medication use and improves migraine-related life impact in subjects with EM and CM. Its clinical

efficacy was observed as early as day 1 and was sustained throughout the study period. It is well-tolerated with a good safety profile in the study population. It has the benefit of intravenous infusion allowing for 100% bioavailability, quicker onset of action, and also offers the advantage of quarterly dosing. The treatment requires the use of an infusion service. Eptinezumab is an attractive addition to the armamentarium of migraine prevention and perhaps even acute treatment.

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Chapter 9
Erenumab

Thien Phu Do, Samaira Younis, and Messoud Ashina

9.1 General

Regulatory approval of erenumab (Aimovig®), previously known as AMG 334, for the preventive treatment of migraine marked the dawn of a new era of mechanism-based migraine medications [1]. Erenumab, the first in its drug class, is a fully human immunoglobulin (Ig) G2 monoclonal antibody (mAb) that targets the calcitonin gene-related peptide (CGRP) receptor with a high potency and selectivity [2]. Erenumab is administered as monthly subcutaneous injections of either 70 or 140 mg. The mean t_{max} is 5.5 days in healthy participants consistent with an early onset of effect within the first week of treatment [3–5]. The plasma half-life is approximately 21–23 days [3, 4]. The efficacy and safety have been established in randomized clinical trials (RCTs) (Table 9.1) and as of June 2020, preventive treatment of migraine with erenumab is launched in more than 38 countries [6] and reports of the first real-world experiences are emerging.

9.2 Compelling Evidence from Randomized Clinical Trials

Two-phase II RCTs suggested that 70 and 140 mg doses of erenumab might be a potential preventive therapy for individuals with episodic or chronic migraine [7, 8]. These results were later confirmed by two pivotal phase III RCTs, STRIVE (NCT02456740) and ARISE (NCT02066415) conducted from 2015 to 2016 [9, 10]. STRIVE and ARISE showed that erenumab was efficacious and tolerable, paving the way for regulatory approval (Tables 9.2 and 9.3) [1, 9, 10].

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_9

Table 9.1 Characteristics of randomized clinical trials of erenumab for the preventive treatment of migraine

Study	Study phase	Patient population	Treatment arms[a]
Sun et al. (2016) [7].	Phase II	Episodic migraine with or without aura ≤2 prior unsuccessful preventive treatments	Placebo Erenumab 7 mg Erenumab 21 mg Erenumab 70 mg
Tepper et al. (2017) [8].	Phase II	Chronic migraine with or without aura ≤3 prior unsuccessful preventive treatments	Placebo Erenumab 70 mg Erenumab 140 mg
Goadsby et al. (2017) (STRIVE) [9]	Phase III	Episodic migraine with or without aura ≤2 prior unsuccessful preventive treatments	Placebo Erenumab 70 mg Erenumab 140 mg
Dodick et al. (2018) (ARISE) [10]	Phase III	Episodic migraine with or without aura ≤2 prior unsuccessful preventive treatments	Placebo Erenumab 70 mg
Reuter et al. (2018) (LIBERTY) [15]	Phase III	Episodic migraine with or without aura 2–4 prior unsuccessful preventive treatments	Placebo Erenumab 140 mg

All studies were multicenter, parallel-group, randomized, double-blinded, placebo-controlled trials
[a]All treatment interventions were administered as monthly subcutaneous injections

Table 9.2 Pooled ≥50% monthly migraine days responder rates for therapeutic dosages 70 and 140 mg in randomized clinical trials of erenumab for the preventive treatment of migraine

	Number of pooled events/participants		
	Erenumab	Placebo	Therapeutic gain (95% CI)
Erenumab 70 mg			
At week 4	223/782 (28.5%)	128/885 (14.5%)	14.0% (10.1%–17.9%)
At week 12	362/881 (41.1%)	277/1029 (26.9%)	14.2% (10.0%–18.4%)
At week 24	147/312 (47.1%)	93/316 (29.4%)	17.7% (10.2%–25.2%)
Erenumab 140 mg			
At week 4	183/624 (29.3%)	87/721 (12.1%)	17.2% (12.9%–21.5%)
At week 12	266/624 (42.6%)	166/721 (23.0%)	19.6% (14.7%–24.6%)
At week 24	156/318 (49.1%)	93/316 (29.4%)	19.7% (12.3%–27.1%)

Modified from: Lattanzi S et al. Erenumab for Preventive Treatment of Migraine: A Systematic Review and Meta-Analysis of Efficacy and Safety. Drugs. doi:https://doi.org/10.1007/s40265-019-01069-1 [35]

Table 9.3 Pooled incidence rates of the most common adverse events in randomized clinical trials of erenumab for the preventive treatment of migraine

Outcome	Number of pooled events/ participants		Risk ratio (95% CI)
	Erenumab	Placebo	
Any adverse event	786/1519 (51.7%)	618/1167 (51.9%)	0.95 (0.88–1.02)
Any serious adverse event	28/1519 (1.8%)	20/1167 (1.7%)	0.96 (0.54–1.71)
Discontinuation due to any adverse event	24/1519 (1.6%)	14/1167 (1.2%)	1.12 (0.57–2.18)
Arthralgia	15/738 (2.0%)	11/472 (2.3%)	0.93 (0.39–2.22)
Back pain	18/858 (2.1%)	13/596 (2.2%)	0.99 (0.47–2.10)
Constipation	28/1294 (2.2%)	11/890 (1.2%)	1.56 (0.73–3.35)
Fatigue	30/1141 (2.6%)	19/885 (2.1%)	1.26 (0.71–2.22)
Hypertension	5/633 (0.8%)	8/319 (2.5%)	0.36 (0.12–1.13)
Influenza	24/1022 (2.3%)	21/761 (2.8%)	0.96 (0.52–1.77)
Injection site pain	49/1413 (3.5%)	23/1014 (2.3%)	1.69 (1.01–2.82)
Migraine	24/1400 (1.7%)	23/1043 (2.2%)	0.78 (0.43–1.39)
Muscle spasm	8/378 (2.1%)	4/282 (1.4%)	1.49 (0.45–4.91)
Nasopharyngitis	107/1519 (7.0%)	83/1167 (7.1%)	0.88 (0.50–1.52)
Nausea	33/1400 (2.4%)	28/1043 (2.7%)	0.92 (0.55–1.54)
Sinusitis	24/916 (2.6%)	13/608 (2.1%)	1.19 (0.60–2.35)
Upper respiratory tract infection	71/1519 (4.7%)	39/1167 (3.3%)	1.26 (0.86–1.85)
Urinary tract infection	12/633 (1.9%)	7/319 (2.2%)	0.86 (0.34–2.17)

Modified from: Lattanzi S et al. Erenumab for Preventive Treatment of Migraine: A Systematic Review and Meta-Analysis of Efficacy and Safety. Drugs. doi:https://doi.org/10.1007/s40265-019-01069-1 [35]

In STRIVE, 955 persons with episodic migraine (mean number of 8.3 ± 2.5 migraine days per month at baseline) were randomized to monthly subcutaneous injections of 70 mg erenumab, 140 mg erenumab or placebo [9]. The primary endpoint was change in the mean number of migraine days per month. Reduction in number of migraine days per month was 3.2 ± 0.2 with 70 mg, 3.7 ± 0.2 with 140 mg vs. 1.8 ± 0.2 days in the placebo group (vs. placebo, $p < 0.001$ for each dose). The responder rate, defined as proportion of participants achieving a ≥50% reduction in the number of migraine days, was 43.3% with 70 mg erenumab (vs. placebo, $p < 0.001$), 50.0% with 140 mg erenumab (vs. placebo, $p < 0.001$) and 26.6% with placebo yielding a therapeutic gain of 17.3% for 70 mg and 23.4% for 140 mg. In ARISE, 577 persons with episodic migraine (mean number of 8.3 ± 2.6 migraine days per month at baseline) were randomized to monthly subcutaneous injections of 70 mg erenumab or placebo [10]. The primary endpoint was change in the mean number of migraine days per month. The erenumab group had 2.9 ± 0.2 days reduction in the number of migraine days per month compared to a 1.8 ± 0.2 reduction in the placebo group (vs. placebo, $p < 0.001$). The responder rate was 39.7% in the erenumab group and 29.5% in the placebo group, yielding a therapeutic gain of

10.2% (p = 0.010). In both RCTs, adverse event rates were similar between the erenumab and placebo groups [9, 10]. In STRIVE, 2.2% (n = 7/317) in the 70 mg erenumab group and 2.2% (n = 7/319) in the 140 mg erenumab group discontinued treatment due to adverse events [9]. In ARISE, 1.8% (n = 5/283) of participants in the erenumab group discontinued treatment due to adverse events [10]. Low discontinuation rates due to adverse events of ~2% in both phase III RCTs demonstrate the good tolerability of erenumab.

9.3 Sustained Efficacy and Safety in Open-Label Extension Studies

Regulatory approval of erenumab was based on results from 3- to 6-month treatment periods. While these trials established robust efficacy and tolerability, erenumab will be used for longer periods than seen in the context of clinical trials. Therefore, it is essential to evaluate whether long-term use of erenumab is sustainable, especially as concerns related to potential vascular safety have been raised [11].

A pooled analysis of four placebo-controlled trials with open-label extensions (up to 3 plus years), with a cumulative exposure of ~2641 patient-years, showed no difference in reports on adverse events between the double-blinded treatment phase and the long-term open-label extensions suggesting a stable adverse event profile over time [12]. Furthermore, a pooled analysis, specifically investigating vascular safety, did not detect a difference between erenumab and placebo groups [13]. An interim analysis of an ongoing five-year open-label extension study, the longest to date, also revealed adverse events rate consistent with the placebo-controlled studies [14]. Collectively, these analyses of open-label extensions demonstrate that prolonged exposure to erenumab is sustainable in terms of efficacy and tolerability for designated patient populations in a clinical trial regime. However, these data do not necessarily reflect real-world treatment, which includes a more heterogeneous patient population with different comorbidities. Future real-world studies will show a more complete picture of tolerability.

9.4 Difficult-to-Treat Patients

A substantial number of candidates for erenumab treatment will be patients who previously failed several preventive medications or report medication overuse.

LIBERTY (NCT03096834), a phase IIIb clinical trial, investigated the efficacy and tolerability of erenumab in patients with episodic migraine with two-to-four previous unsuccessful migraine preventives [15]. In this RCT, 246 participants were randomized to erenumab 140 mg or placebo. At week 12, the responder rate was 30% in the erenumab group vs. 14% in the placebo group (p = 0.002). The responder

rate is lower compared to other RCTs with a more treatment-naive population (30% vs. 49.1% for pooled responder rate at week 24; Table 9.2), however, consistent with post hoc and subgroup analyses of erenumab and other anti-CGRP mAbs [16, 17]. Altogether, erenumab and other drugs targeting the CGRP pathway seem to work in patients with previous unsuccessful preventive treatments [16–18].

Patients with chronic migraine commonly overuse acute medications, which can lead to medication-overuse headache [19, 20]. A recent RCT suggested that preventive treatment in patients with medication-overuse headache is beneficial [21]. A subgroup analysis of a phase II RCT showed that patients with chronic migraine and concurrent medication-overuse headache (41%; n = 274/667 patients) treated with 70 or 140 mg erenumab had a higher responder rate than the placebo group at week 12 (erenumab 70 mg: 36%; erenumab 140 mg: 35%; placebo: 18%) parallel with reduction in acute medication use days [22]. These results suggest that clinical benefit of erenumab is not necessarily limited by acute headache medication overuse. As such, a phase IV clinical trial is currently investigating whether erenumab is effective and tolerable in adults with chronic migraine with at least one preventive treatment failure and diagnosed with medication-overuse headache (NCT03971071).

9.5 Postmarketing Safety Data

There have been notable safety updates related to constipation and hypertension after erenumab was approved for treatment [23].

Constipation was one of the most common adverse events in clinical trials and rated mild to moderate in severity (Table 9.3) [9, 10]. In the postmarketing setting, constipation with serious complications including hospitalization and surgery have been reported following the use of erenumab [23]. Most of these serious reactions occurred already following the first dose administration. Concomitant medication associated with constipation may increase the risk of severe cases [23].

Development and worsening of hypertension have been reported in the postmarketing setting including cases requiring pharmacological intervention and hospitalization [23]. Like constipation, hypertension was most frequently reported after the first dose administration. This contrasts a post hoc safety analysis of clinical trials where the reports of hypertension were comparable between active and placebo (placebo: 0.9%; erenumab 70 mg: 0.8%; erenumab 140 mg: 0.2%), however, high cardiovascular risk patients were excluded from these trials [13].

The emerging safety data surrounding constipation and hypertension do not necessarily suggest that patients with these comorbidities or risk factors should be categorically excluded from treatment with erenumab. However, increased screening and monitoring of high-risk patients is warranted. This is especially relevant for constipation as persons with migraine may have a higher prevalence of comorbid gastrointestinal disorders including constipation in comparison to the general population [24, 25]. Furthermore, constipation is one of the most common adverse event-related reasons for treatment discontinuation in a real-world setting [26]. Potential

discontinuation of erenumab due to complications should still be evaluated on a case-by-case basis until more data are available.

9.6 Real-World Clinical Experience

Preliminary observational data from one Italian center suggested that erenumab is effective in patients with episodic or chronic migraine, although data only cover up to 8 weeks of treatment [27]. The ≥50% responder rates were 50% in the episodic migraine cohort and 68.2% in the chronic migraine cohort at week 4. Adverse event rate was not reported. The second published paper included observational data from multiple Italian centers with difficult-to-treat patients, here defined as persons with long history of disability due to headache/migraine and previous treatment failures [28]. Responder rate was reported to be higher than in RCTs with 62.9% patients being responders during the first 3 months of treatment. The most common adverse event was constipation occurring in 13.5% of patients. Only one patient discontinued treatment due to adverse events, an allergic reaction. Real-world data of patients with refractory chronic migraine from a center from the United Kingdom revealed a ≥50% responder rate of 35% at month 3 [26]. Constipation was the most frequent adverse event and reported by 20% of participants. Twelve percent of participants discontinued treatment due to adverse events, primarily constipation (9/19 dropouts).

Interestingly, responder rates in the Italian studies were higher than those reported in RCTs (Table 9.2), which may be owed to the fact that real-world data are not placebo-controlled. Cautious interpretation should therefore be exercised until long-term real-world data are available. The frequency and distribution of adverse events are mostly similar to RCT data, except for the higher occurrence of constipation of 13.5% in the multicenter Italian study and 20% in the United Kingdom study versus 2.2% of the pooled incidence rate of RCTs (Table 9.3).

9.7 Future Perspectives

Considering the high costs of erenumab and restricted availability due to different reimbursement schemes, the next step is identifying predictors of treatment response, while paving the way for precision medicine in migraine [29]. This is especially pertinent as a subpopulation of patients treated with erenumab achieves ≥75% reduction in monthly migraine days, so-called super-responders [30]. In this context, experimental human headache models may potentially be used as a biomarker to predict the efficacy of CGRP-targeting therapies [31]. The hypothesis is that patients who develop a migraine attack after a CGRP challenge will benefit more from erenumab or other anti-CGRP mAbs. Indeed, a proof-of-concept study with 13 patients previously treated with erenumab reported that treatment

responders were highly susceptible to CGRP provocation [32], but larger studies are needed to confirm these findings.

Moreover, the use of erenumab for other indications such as other headache or pain disorders may be a possibility. In a small case series, erenumab had an effect on comorbid cluster headache (a primary headache disorder belonging to the group of trigeminal autonomic cephalalgias) in patients with migraine [33]. A 12-week open-label study of erenumab for the preventive treatment of persistent post-traumatic headache (a secondary headache disorder), attributed to mild traumatic brain injury, reported a lower frequency of moderate to severe headache days, which often mimic the features of a migraine-like headache [34]. In both cases, RCTs are needed to confirm these findings. An RCT evaluating the efficacy and tolerability erenumab for trigeminal neuralgia, a disorder characterized by recurrent unilateral brief electric shock-like pains limited to the divisions of the trigeminal nerve, is also underway (NCT04054024).

Glossary

ARISE (NCT02066415) A randomized, double-blind, placebo-controlled, parallel-group, phase 3 trial of erenumab 70 and 140 mg administered monthly in adults with episodic migraine.

CGRP Calcitonin gene-related peptide.

Gepant Small-molecule calcitonin gene-related peptide receptor antagonist.

Ig Immunoglobulin.

LIBERTY (NCT03096834) A randomized, double-blind, placebo-controlled, parallel-group, phase 3b trial phase IIIb of erenumab 140 mg administered monthly in adults with episodic migraine with two-to-four previous unsuccessful migraine preventives.

mAb Monoclonal antibody.

RCT Randomized clinical trial.

Responder rate Percentage of patients achieving a reduction of 50% or more monthly number of migraine days.

STRIVE (NCT02456740) A randomized, double-blind, placebo-controlled, parallel-group, phase 3 trial of erenumab 70 and 140 mg administered monthly in adults with episodic migraine.

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Chapter 10
Fremanezumab

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10.1 Introduction to the Compound

Fremanezumab (AJOVY®) has been the second FDA-approved humanized monoclonal antibody for the treatment of migraine, in September 2018. Moreover, it received the EU market authorization in March 2019. In 2020, the National Institute for Health and Care Excellence (NICE) has issued a positive opinion regarding the use of AJOVY (Fremanezumab) as preventative chronic migraine drug in the Final Appraisal Determination (FAD). NICE recommends the administration of AJOVY in patients with chronic headaches who have not responded to at least three previous drug prophylactic treatments.

10.2 Chemistry

The chemical name of Fremanezumab (synonyms TEV-48125, LBR-101, PF-04427429, RN307) is Immunoglobulin G2, antihuman alpha calcitonin gene-related peptide/beta calcitonin gene-related peptide (CGRP) [1]. Fremanezumab is a humanized immunoglobulin G2 (IgG2) Δa monoclonal antibody (mAb) derived

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_10

from a murine precursor with a molecular weight of approximately 148 kDa. It has been mutated in the Fab variable region of the heavy chain to increase affinity and to limit antibody Fc effector function. Moreover, two mutations (A330S and P331S) were introduced into the constant region of the heavy chain to limit antibody Fc effector function (ADCC and CDC). It is administered subcutaneously with a quarterly dosing of 675 mg as three 225 mg SC injections every 3 months, while the monthly dosing is 225 mg SC injection each month [1].

10.3 Fremanezumab Pharmacodynamics and Pharmacokinetics

Fremanezumab shows typical pharmacokinetic features of other therapeutic antibodies. Fremanezumab targets both α and β-CGRP isoforms, preventing their binding to CGRP and AMY1 receptors. Fremanezumab can target specifically CGRP ligand and it is unclear if fremanezumab is also able to bind AM or other human calcitonin receptors family compounds [2]. A phase I clinical trial conducted in order to assess the pharmacokinetics of fremanezumab different subcutaneous doses on Japanese and Caucasian healthy subjects showed that C_{MAX} were 0.91, 1.04, and 1.14 for 225 mg, 675 mg and 900 mg doses, respectively [3]. Across doses, mean T_{MAX} was the same for both ethnicities for 225 and 675 mg doses (7 and 5 days) and similar for Japanese and Caucasian subjects at the 900 mg dose level (11 and 7 days, respectively). Plasma concentrations of fremanezumab reach the maximum peak (C_{MAX}) and overall (all AUCs) within 5–7 days for all three doses administered. As expected, plasma concentrations increased with increasing doses, with gradual decline thereafter. In both ethnic study subject groups, subcutaneous fremanezumab, binding to protective receptors as the other IgG2 molecules, has a long mean half-life ranging from 31 to 39 days in both Japanese and Caucasian groups [3]. However, a further Phase I program [4] assessed that half-life of fremanezumab was 45 days in healthy volunteers. Across all the three administered doses, both CL/F (0.08–0.09 mL/min) and Vz/F (5.71–6.43 L) were similar, suggesting minimal distribution to the extravascular tissues. However, data regarding the protein binding of fremanezumab were not available. As all other monoclonal antibody agents, hepatic or renal impairment is not expected to affect fremanezumab pharmacokinetics, even if no study has included patients affected by hepatic or renal disorders. Nevertheless, monoclonal antibody agents like fremanezumab are not eliminated via hepatic, renal, or biliary routes, but they are known to be mainly eliminated via intracellular enzymatic proteolysis, producing small peptides and amino acids [2]. For these reasons fremanezumab, being an anti-CGRP mAb, exhibits PK/PD advantages of target specificity, prolonged half-lives, reduced potential for hepatotoxicity and limited drug–drug interactions [5, 6].

10.4 Fremanezumab Clinical Efficacy

The clinical efficacy of fremanezumab as a preventative treatment for episodic and chronic migraine conditions was assessed in two multicenter, randomized placebo-controlled, 3-month studies. In the study conducted for the evaluation of fremanezumab clinical efficacy in episodic migraineurs, 875 patients were enrolled and divided (1:1:1) into three groups: 290 patients were assigned to the AJOVY 225 mg monthly group, 291 patients were administered with AJOVY 675 mg quarterly (an initial dose of 675 mg and then two placebo doses) and 294 patients received the placebo dose monthly. The study has included patients using additional prophylactic drugs (21% of patients), while excluded patients affected from several pathological conditions, such as cardiovascular disease, deep vein thrombosis, transient ischemic attack, or other vascular or thrombotic events. The primary endpoint was the reduction of the monthly migraine days at 9–12 weeks from baseline [7]. During the 12 weeks after the first dose, the least-square mean (LSM) change value in migraine days per month was significantly reached for both dose regimens, resulting in 4.9 days for the monthly dosing group (LSM change from baseline of −3.7 days), 5.3 days for higher dosing (LSM change from baseline of −3.4 days), and 6.5 days for the placebo group (LSM change from baseline of −2.2 days). Thus, compared to placebo, both fremanezumab 225 mg and 625 mg were effective in reducing the mean number of migraine days (1.5 and 1.3 days respectively, $p < 0.001$), over the 3-month period. Secondary endpoints included the following: (i) ≥50% reduction in mean migraine days per month; (ii) the reduction of any acute migraine drugs from baseline to 12 weeks; (iii) mean migraine days per month; (iv) mean monthly migraine days in patients not receiving concomitant prophylactic treatments for migraine; (v) mean change in Migraine Disability Assessment (MIDAS) score. The study showed a 50% decrease of the monthly migraine days (at week 12) in 47% of subjects administered with fremanezumab 225 mg monthly, in 44.4% of subjects administered with a single 675 mg injection, and in 27.9% of subjects receiving placebo. The migraine days per month requiring an additional acute treatment were 4.4 for the monthly dosing group (LSM change from baseline, −3.0 days), 4.6 for 675 mg dose (LSM change from baseline, −2.9 days), and 5.8 in placebo group (LSM change from baseline, −1.6 days). Moreover, the MIDAS score reduced significantly in fremanezumab-treated groups. In particular, after 4 weeks from the administration, MIDAS decreased from 38 to 12.6 in the monthly dosing group, from 47.1 to 14.6 in the quarterly dosing group, and from 37.3 to 19.4 in the placebo group. A total of 791 patients completed the 3-month double-blind phase [7].

Another study [8], aimed to evaluate the efficacy of Fremanezumab in patients with chronic migraine, randomized 1130 patients (headache occurring on ≥15 days, with characteristics of migraine headache on ≥8 days) in two different groups: 375 patients received a 675 mg starting dose followed by 225 mg monthly, 375 patients received a single dose of 675 mg every 3 months (quarterly) and 371 patients were administered with placebo. The primary endpoint of this study was the reduction of

the mean number of headaches per month from baseline. The results showed a decrease of 4.3 days for the quarterly dosing group, 4.6 for monthly group, and 2.5 in the placebo dosing. The secondary endpoints of the study consisted of: (1) a decrease of the mean number of monthly migraine days, significantly improved in all three groups; (2) a reduction of at least 50% of the mean headache days and this was achieved in 38, 41, and 18% of patients of the quarterly, monthly and the placebo groups respectively ($p < 0.001$ for both comparisons with placebo); (3) a change from baseline in monthly average number of days of additional acute headache treatment (−4.2, −3.7, −1,9 for 225 mg, 675 mg, placebo respectively); (4) the reduction in the 6-item Headache Impact Test (HIT-6) score, that resulted significantly decreased in fremanezumab quarterly (6.4 ± 0.5 points) and fremanezumab monthly (6.8 ± 0.4 points) groups with respect to placebo (4.5 ± 0.5 points; $p < 0.001$ for both dose regimens). A total of 1034 patients completed the 3-month double-blind phase [8].

10.5 Fremanezumab Safety and Tolerability

Preclinical and clinical studies demonstrated that fremanezumab does not determine changes in vital signs or laboratory tests (including liver enzymes); also, cardiac electrical conduction or repolarisation and other important changes in the ECG parameters were not observed [9]. However, AJOVY may cause allergic reactions in the injection site, including itching, rash, and hives that can happen within hours and up to 1 month after receiving AJOVY. In fact, it is contraindicated in subjects with severe hypersensitivity to fremanezumab-vfrm or to any of the other prefilled syringe excipients. Adverse reactions were reported by ≥2% of patients on AJOVY and greater than placebo. Only less than 2% of the subjects discontinued AJOVY because of side effects [7, 8].

Belonging to the therapeutic protein drug family, AJOVY has a potential for immunogenicity. In order to assess this clinical parameter, AJOVY was monitored by analyzing antidrug antibodies (ADA) and also the neutralizing antibodies in the treated patients. The results on the first 3 months showed that treatment-emergent ADA responses were observed in 0.4% of the sample. Only one patient developed neutralizing antibodies at day 84. ADA responses increased up to 1.6% of the sample in the long-term treatment. Of these 1.6% of patients developing ADA, 17 also had a neutralizing activity in their post-dose samples. Further studies are needed to assess if this fremanezumab-vfrm antibody development affects efficacy and safety of AJOVY [2].

Regarding pregnancy and lactation, there are no adequate data on the risks related to the use of AJOVY in pregnant women and on the presence of fremanezumab-vfrm in human milk. Also, for paediatric and geriatric use data on safety and efficacy are not available.

10.6 Ongoing Trials

Up to date, several clinical trials are ongoing to assess the efficacy and safety of fremanezumab in different pathological conditions.

One study is investigating the efficacy of fremanezumab in reducing pain in patients with interstitial cystitis–bladder pain syndrome (IC-BPS). A secondary objective of the study is to evaluate the effect of fremanezumab on other efficacy measures, including pain, voiding frequency, urinary symptoms, and quality of life. Another secondary objective of the study is to evaluate the safety and tolerability of fremanezumab administered subcutaneously in adult patients with IC-BPS [10].

A second study is investigating side effects of fremanezumab when treating patients with Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) for migraine headaches. Primary outcomes are changes in migraine-related disability, headache intensity, and the evaluation of adverse event risks [11].

A third study is testing if Fremanezumab is effective in preventing chronic and episodic migraine in patients 6–17 years of age. The primary objective of this study is to evaluate long-term safety and tolerability, while the secondary objectives are to assess the efficacy of subcutaneous fremanezumab in pediatric migraineurs and to evaluate the immunogenicity of the drug and the impact of ADAs on clinical outcomes [12].

Another study is evaluating safety and efficacy of fremanezumab for the preventative treatment of migraine patients suffering from major depressive disorder. The primary outcome is the mean change in monthly average number of migraine days, while the main secondary outcomes are mean changes in depression symptoms, quality of life, and the evaluation of the occurrence of adverse events in patients taking concomitant medications [13].

A study is investigating the effectiveness of fremanezumab administered subcutaneously in reducing pain in adult patients with fibromyalgia. A secondary objective of this study is to assess the effect of fremanezumab on efficacy measures such as pain, quality of life, sleep, fatigue, improvement in health, physical functioning, and mood. Another secondary objective is to evaluate the safety and tolerability of fremanezumab administered subcutaneously in adult patients with fibromyalgia [14].

10.7 Conclusion

Fremanezumab, a humanized monoclonal antibody, inhibits the interaction of CGRP with its receptor. FDA and EMA approved its clinical use for migraine prevention in adults. The available results from two Phase II and two Phase III randomized clinical trials showed a good efficacy of treatment with subcutaneous fremanezumab for episodic and chronic migraine with respect to placebo. Adverse

events were mild or moderate and related to the injection site reactions (erythema, pain, or induration), but they occurred relatively frequently. Vital signs, laboratory findings, and other clinical parameters did not show relevant changes. Several other clinical trials are actually ongoing with the aims of evaluating fremanezumab efficacy for prevention of episodic and chronic cluster headache and post-traumatic headache. Although long-term safety is still being evaluated in an ongoing trial, fremanezumab represents a potentially useful option for the management of migraine disease.

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Chapter 11
Potential Side Effects and Pregnancy

Eloísa Rubio-Beltrán

11.1 Introduction

Migraine is a highly disabling neurovascular disorder [1]. It is estimated that 16% of the world population suffers from migraine, being two to three times more prevalent in women than in men, suggesting a role for sex hormones [2, 3].

As mentioned in the previous chapters, calcitonin gene-related peptide (CGRP) has been described to play an important role in migraine pathophysiology [4, 5], which led to the development of *gepants*, CGRP receptor antagonists (e.g., olcegepant and telcagepant) for the acute treatment of migraine. Unfortunately, even though gepants were shown to be effective [6, 7], they did not reach the market due to pharmacokinetic limitations and hepatotoxicity reports [8]. It is worth mentioning that, currently, novel (non-structurally related) gepants have been developed for the acute and prophylactic treatment of migraine, most of them already approved and with no hepatotoxicity reports so far [9–11].

In order to discard the hepatotoxicity concerns, CGRP (receptor)-antibodies were developed for the prophylactic treatment of migraine [12–14], all have been shown to be effective and no serious side effects have been reported [15]. However, CGRP is widely expressed throughout the body, therefore it is important to consider the physiological role of this peptide and the possible side effects after long-term blockade of the CGRP pathway. Moreover, migraine is more prevalent in women, and interactions between the CGRP pathway and sex hormones have been previously reported [3], thus the possible concerns regarding patients in child-bearing age should be addressed. In this chapter, the potential side effects after long-term blockade of the CGRP pathway will be discussed. Additionally, the possible concerns regarding the use of CGRP (receptor) antibodies in patients in child-bearing age will be discussed.

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_11

11.2 CGRP and (Patho)Physiology

During the last decades, studies have shown that CGRP not only plays an important role in migraine pathophysiology but also in physiological processes and the homeostatic response during pathophysiological conditions [16, 17](Fig. 11.1). As migraine patients present an increased risk of myocardial infarction, coronary artery disease, hemorrhagic and ischemic stroke, and altered arterial function, with women being at higher risk [18–26], this chapter will emphasize the role of CGRP in the cardiovascular system.

11.2.1 CGRP and Cardiovascular System

In the cardiovascular system, CGRPergic fibers have been shown to innervate the blood vessels and the heart [27–29]. Accordingly, it has been shown that CGRP participates in the regulation of blood pressure [30, 31], with intravenous administration of CGRP resulting in a decrease of systolic and diastolic blood pressure and an increase of heart rate [32]. However, these modulatory roles seem to be rather limited in physiological conditions [30]; instead, CGRP seems to participate as a protective and/or compensatory mechanism in hypertension. Studies with murine models of hypertension have shown that in CGRP knockout mice there is a significant increase in mean arterial pressure, renal damage, and aortic hypertrophy, when compared to wild types [31, 33, 34]. Additionally, an upregulation of the CGRP-receptor components expression has also been described in models of hypertension, which reinforces the participation of the CGRP pathway as a compensatory mechanism [31]. Studies have also shown that the increase in mean arterial pressure correlates with an increased activation of the sympathetic system [34, 35] and that during chronic hypertension, CGRP is involved in the maintenance of cerebrovascular reactivity [36]. Although none of the clinical trials with the CGRP (receptor)-antibodies have reported changes in blood pressure so far [15], the duration of these trials is not sufficient to see the long-term vascular effects of continuingly blocking CGRP or its receptor [37]. Interestingly, one case of new-onset Raynaud's phenomenon (RP) while taking erenumab and two cases of patients with exacerbated RP while taking fremanezumab and galcanezumab have been reported [37]. While RP is more common in migraine patients when compared to the general population, it is possible that CGRP blockade may be involved in the exacerbation of RP as in patients with primary RP a significant reduction in CGRP immunoreactive neurons in the skin of the fingers has been described [38, 39]; therefore, it is important to be cautious when prescribing CGRP (receptor)-antibodies to patients with established RP.

Besides its protective role during hypertension, CGRP has been shown to be involved in the hemodynamic and metabolic changes in response to ischemic events [40–46]. In patients with congestive heart failure, it has been shown that

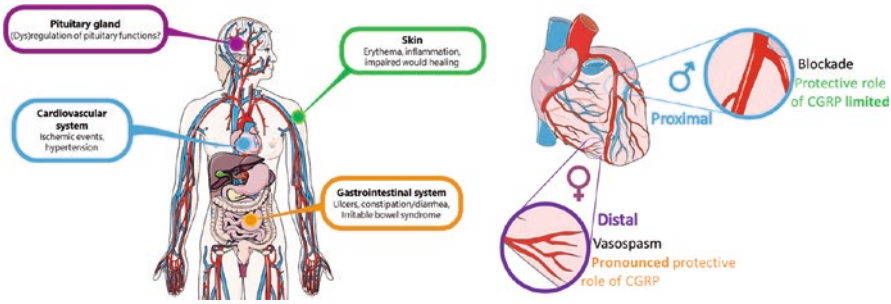

Fig. 11.1 Theoretical concerns after long-term exposure to CGRP (receptor)-antibodies. An overview of the organ systems where CGRP and the receptor are present and possible side effects that could be caused by the nonselective blockade of α- and β-CGRP with the CGRP (receptor)-antibodies (left). In myocardial ischemia, CGRP seems to have a more prominent role in the distal portion than in the proximal portion of the coronary arteries, which may represent a downside for women, as ischemic events in the distal portion are more common in female patients, while proximal obstructions are more prevalent in male patients (right). Modified from [12] (licensed under the terms of the Creative Commons Attribution 4.0 International License http://creativecommons.org/licenses/by/4.0/) and from [55], with permission

infusion of CGRP results in an increase of myocardial contractility [47], and lower levels of CGRP in plasma have been described in patients with established coronary artery disease [48]. Furthermore, preclinical studies in models of ischemic stroke have shown that when CGRP is administrated at the beginning of reperfusion there is a reduction in brain edema, probably associated to a decrease in the blood–brain barrier disruption [49], with an enhanced CGRP-dependent vasodilation also described [50]. Accordingly, in patients with subarachnoid hemorrhage, plasma, and cerebrospinal fluid levels of CGRP inversely correlate with vasospasm [51, 52] and infusion of CGRP further reduces vasospasm [53]. While no preclinical studies with the CGRP (receptor)-antibodies have been performed in murine models of stroke, a recent study in mice treated with the CGRP receptor antagonists olcegepant and rimegepant before middle cerebral artery occlusion observed that gepants reduced collateral flow and reperfusion success, resulting in worsened ischemic stroke [54].

A crucial aspect to consider when studying the impact of long-term blockade of CGRP in the cardiovascular system is the role of sex hormones in the modulation of CGRP signaling and in the presentation of ischemic events, especially considering the high prevalence of migraine in women [2, 3, 55]. To begin with, CGRP signaling seems to be modulated by ovarian steroid hormones, with women having higher plasma CGRP levels than men, and the levels further increase when patients are under contraceptives [56]. Moreover, studies have shown that when given simultaneously with 17β-estradiol or progesterone, the CGRP-dependent decrease in blood pressure and the increase in myocardial contractility are enhanced [57, 58], suggesting a synergistic interaction between ovarian steroid hormones and CGRP. Also, female migraine patients have a higher risk of stroke when compared

to men with migraine, and the prevalence of (cardio)vascular events rises sharply after menopause [59, 60]. This sharp increase may be due to the increase in plasma cholesterol and triglyceride levels in postmenopausal women when compared to premenopausal women [61]. Accordingly, an increase in CGRP expression in adipose tissue of postmenopausal women has been described, possibly as a protective mechanism against cardiovascular events [62]. Furthermore, in myocardial infarction, women present angina-like chest pain, but no visible obstructions during angiography as it is most likely caused by vasospasms of the small intramyocardial portions of the coronary arteries. In contrast, men are more likely to present visible occlusions of the proximal conducting portion [63, 64] (Fig. 11.1). As the cardioprotective role of CGRP in coronary arteries has been described to be more prominent in the (small) distal portions that in the proximal portions, this suggests that women treated with CGRP (receptor)-antibodies may be at greater risk than men during a cardiac ischemic event especially after menopause [17, 65, 66]. Furthermore, *in vitro* studies in human coronary arteries have shown that administration of erenumab inhibits the CGRP-dependent relaxations more potently and efficacious in the distal portion that in the proximal portion [67]. Interestingly, out of the four CGRP (receptor)-antibodies currently available, only erenumab has explored the cardiovascular safety of CGRP receptor blockade in patients with stable angina during a treadmill test, with no differences found between the erenumab and placebo group [68], suggesting that CGRP (receptor)-antibodies are safe in patients with a history of cardiovascular events. However, as mentioned above, the sex differences in migraine prevalence and the cardioprotective role of CGRP are a crucial aspect to consider when studying the impact of long-term CGRP blockade [69]. Besides the pharmacokinetic limitations of this study, such as the lack of long-term effects exploration since only a single administration of erenumab was investigated, the low biodistribution as the treadmill test took place 30 min after infusion of erenumab and the lack of evidence of CGRP receptor blockade, the main pitfall of this study was the high percentage of male patients. As discussed above, migraine is more prevalent in women, and myocardial infarction in women is more commonly associated to vasospasm of the small intramyocardial parts of the coronary arteries [63] that are more densely innervated with CGRPergic fibers than the proximal arteries. This difference in pathophysiology could mean a difference in risk for men and women when blocking CGRP. Therefore, there is an urgency for safety studies with a different design, including the consideration of gender differences. Recently, a case report of a 41-year-old woman with migraine without aura who developed a right thalamic infarction during a typical migraine attack after 1 month of her first dose of erenumab (70 mg) has been published. Imaging studies suggested vasospasm of the right posterior cerebral artery as probable cause, and further evaluation revealed no specific cause of stroke or vascular risk factors aside from long-term use of oral contraceptive pills and the use of rizatriptan as acute treatment [70]. These results reinforce the importance of *appropriately* addressing the (cerebro)vascular safety of long-term blockade of the CGRP pathway, especially in patients at increased risk of ischemic events (i.e., women on contraceptives).

11.2.2 CGRP, Inflammation, and Wound Healing

CGRP has been described to participate in several inflammatory processes [71–73]. In fact, preclinical studies have explored the use of CGRP antibodies for the treatment of osteoarthritis-related pain [73]. However, CGRP has also been reported to participate in wound healing [74], possibly through the reduction of TNF-α expression and macrophage infiltration [75], increase of keratinocyte proliferation [76], and the facilitation of revascularization by upregulating the expression of vascular endothelial growth factor [77]. Thus, continuous blockade of the CGRP pathway could result in alterations in wound healing. So far, clinical trials have only reported erythema at the site of injection, but this has also been reported in the placebo groups [15]. Nonetheless, recently a case report described a 51-year-old female migraine patient effectively treated with erenumab for 6 months, with two periods of severely impaired wound healing after a minor skin injury. A skin biopsy confirmed the presence of deep perivascular and interstitial lymphohistiocytic infiltrate with admixed eosinophils, ulceration of the epithelium, edema of the papillary dermis, and focally thrombosed vessels [78]. Therefore, it is important to be aware of the possibility of impaired healing after minor lesions and, most importantly, after surgical procedures.

11.2.3 CGRP and the Gastrointestinal System

As discussed in previous chapters, there are two isoforms of CGRP: α-CGRP and β-CGRP. While α-CGRP is the isoform involved in migraine pathophysiology and cardiovascular homeostasis, the gastrointestinal tract is highly innervated by β-CGRPergic fibers [79, 80]. The antibodies against CGRP do not distinguish between the α and β isoforms, therefore, the CGRP (receptor)-antibodies also inhibit β-CGRP signaling in the gastrointestinal system. Accordingly, data from the FDA Adverse Event Reporting System show that 17% of the total reported side-effects to the CGRP (receptor)-antibodies are related to the gastrointestinal system [81]. Preclinical studies have shown that β-CGRP participates as a "gastroprotector" in the maintenance of mucosal integrity and ulcer healing, as inhibition of this pathway results in severe mucosal damage [82–84] and may result in the development of inflammatory bowel disease. Moreover, β-CGRP also participates in the modulation of gastrointestinal motility in a biphasic manner, suggesting that patients under CGRP (receptor)-antibodies treatment may present episodes of constipation and diarrhea [85, 86]. In fact, it is estimated that 3–4% of the patients under treatment with CGRP (receptor)-antibodies have reported constipation [81, 87]. This was recently addressed in a double-blind, crossover study where CGRP was infused for 2 hours, and participants were asked about their gastrointestinal symptoms. A total of 93% (27/29) of the patients reported symptoms such as rumbling, stomach pain, nausea, diarrhea, and an urge to defecate [88]. Additionally, a case was

published of a 39-year-old woman with a history of migraine with and without aura, effectively treated with erenumab (70 mg) that initially developed mild constipation and, after open abdominal surgery, developed paralytic ileus, which spontaneously recovered within the following month. Due to the possible role of erenumab in this event, the patient discontinued her treatment and no longer presented mild constipation [89, 90]. Taking into consideration the current evidence, gastrointestinal symptoms are the most prevalent side effects associated to CGRP blockade, and careful monitoring of possible complications is advised.

11.2.4 CGRP and the Central Nervous System

One advantage of the CGRP (receptor)-antibodies is the low brain penetration, thus excluding (most of the) central side effects. However, it is important to consider the structures of the central nervous system that are not protected by the blood–brain barrier, such as the pituitary [91], suggesting a possible modulation of the hypothalamo-pituitary tract functions [92]. Moreover, even though it has been shown that blood–brain barrier permeability is not modified during migraine attacks [93, 94], clinicians should be cautious when prescribing CGRP (receptor)-antibodies to patients with compromised blood–brain barrier, such as in cerebral proliferative angiopathy. A recent case report described a 22-year-old chronic migraine patient with cerebral proliferative angiopathy that presented to the hospital in status epilepticus 2 days after his first dose of erenumab. Imaging studies showed progressive areas of diffusion restriction including the brain tissue adjacent to the cerebral proliferative angiopathy, bilateral white matter, and hippocampi. Six months later, his magnetic resonance showed white matter injury, encephalomalacia surrounding cerebral proliferative angiopathy, and bilateral hippocampal sclerosis. Unfortunately, the patient remains clinically affected with residual symptoms, including refractory epilepsy and cognitive deficits [95]. Thus, even though central side effects are unlikely, close monitoring of pituitary function and discarding patients with preexisting conditions that may facilitate blood–brain barrier permeability are recommended.

11.2.5 CGRP and Other Receptors

As discussed in Chap. 2, CGRP not only binds to its canonical receptor but also to the AMY_1 receptor. Similarly, amylin and adrenomedullin can bind to the canonical CGRP receptor [96, 97]. This suggests that when CGRP receptor is blocked, CGRP can still act through the AMY_1 receptor, thought to also be expressed in human coronary arteries, which may confer a cardiovascular safety advantage. Conversely, in the case of the CGRP blocking antibodies, amylin and adrenomedullin may act on the canonical CGRP receptor, which could theoretically result

in a safety advantage. More studies are required to investigate the possible role of these additional signaling pathways in the efficacy and safety of CGRP (receptor)-antibodies.

11.3 CGRP and Pregnancy

During pregnancy, there are several hemodynamic changes, for instance, a decrease of the uterine vascular resistance and an increase of the uteroplacental blood flow are observed [98]. Interestingly, studies have shown that in healthy pregnant women, CGRP levels are significantly higher throughout the pregnancy reaching their maximum during the last trimester, and the levels later decrease to levels similar to controls in the postpartum phase [99, 100], suggesting that CGRP participates in the regulation of the fetoplacental vascular tone [101, 102]. Conversely, in patients with impaired uteroplacental circulation CGRP levels are lower compared to normotensive pregnancies [103].

Even though CGRP plasma levels are higher in healthy pregnant patients, studies have reported a decrease in migraine attacks during pregnancy and puerperium [3, 99]. The exact mechanism behind this "paradox" is not yet clear, but it is thought that it may be due to a desensitization of the CGRP receptor or to a difference between local cranial *versus* systemic CGRP levels [3]. Furthermore, as previously described, it is crucial to always consider the role of sex hormones. In nonpregnant migraine patients, the (cyclic) decrease in estradiol levels has been associated to an increase in migraine attacks; this decrease is not observed during pregnancy as estradiol levels remain elevated, which could explain the reduction in migraine days during pregnancy [98].

11.3.1 Pregnancy and CGRP (Receptor)-Antibodies

Due to the important role of CGRP in the vascular adaptations during pregnancy, no studies have addressed the efficacy of CGRP (receptor)-antibodies in pregnant patients; therefore, there is not much known regarding their potential side effects. Even though migraine attack frequency decreases during pregnancy, and it is unlikely to prescribe CGRP (receptor)-antibodies to pregnant patients, it is important to consider the possibility of an unplanned pregnancy in a patient under treatment with one of the antibodies. Since the half-life of these antibodies is ~1 months [104], and it would take about five times $t_{1/2}$ before the drug has disappeared from the circulation, there is a theoretical risk for CGRP blockade that could lead to hypertension and fetal growth restriction. Preclinical studies in rats have shown that CGRP receptor blockade during pregnancy results in fetal growth retardation, an increase in systolic blood pressure and fetal mortality [105]. In a murine model of preeclampsia, a pregnancy disorder characterized by high blood pressure and proteinuria, administration of

CGRP reduced blood pressure and fetal mortality [106]. Nonetheless, in a study with primates (*Macaca fascicularis*, cynomolgus monkey), erenumab was administered during pregnancy and no effects on pregnancy, embryo-fetal, or postnatal growth and development were observed [107]. In conclusion, clinicians should take these risks into consideration, together with the long half-life of the CGRP (receptor)-antibodies, when prescribing these drugs to migraine patients of childbearing potential.

11.4 Conclusion

The novel CGRP (receptor)-antibodies for the prophylactic treatment of migraine represent a milestone in migraine therapy. However, it is important to consider that CGRP is not only involved in migraine pathophysiology but also in several physiological processes. In the cardiovascular system, for example, CGRP plays a pivotal role in the homeostatic response to ischemic events. As migraine patients present higher cardiovascular risk (with women at higher risk), chronic blockade of the CGRP pathway poses a concern. Moreover, CGRP also participates in the regulation of the vascular tone during pregnancy; therefore, special caution should be taken when prescribing CGRP (receptor)-antibodies to women of childbearing potential. Additionally, gastrointestinal symptoms and impaired wound healing could be present, thus careful monitoring of these symptoms should be taken. Most importantly, correctly designed studies that address the potential risks are urgently needed.

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Chapter 12
Real-World Data, Clinical Practice So Far

Eleonora De Matteis, Raffaele Ornello, and Simona Sacco

12.1 Introduction

The advent of monoclonal antibodies (mAbs) targeting the calcitonin gene–related peptide (CGRP) has substantially renovated migraine prevention, adding a new, specific, effective, and tolerable treatment to the wide and old armamentarium available [1]. The aforementioned qualities have ensured these drugs a competitive role in both episodic (EM) and chronic migraine (CM) prevention. Traditionally, the sole preventative exerting part of its action on the CGRP pathway was onabotulinumtoxinA [2], which is licensed only for CM prevention.

Since 2018, mAbs have undergone several clinical trials comparing their efficacy and safety with placebo [3]: confirming the promising trial results in clinical practice would be the next challenge for researchers. In terms of overall drug efficacy, recently published real-life studies showed results superior or comparable to clinical trials even in difficult-to treat patients. A search on the PubMed database was completed using keywords and MeSH (Medical Subject Headings) terms for the concepts "real-word," "real-life," "erenumab," "fremanezumab," "eptinezumab," "galcanezumab," and "migraine." A total of 11 studies involving a minimum of ten patients and seven case reports have been evaluated; their design, results, and main characteristics are summarized in the following sections.

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_12

12.2 Design of the Observational Studies

All considered studies were observational retrospective or prospective open-label researches published between 2019 and 2020. They all took place in European countries except for two studies [4, 5] undertaken in the United States. While a preliminary study on galcanezumab has been recently published [6], the other works focused on the efficacy of erenumab in real-life settings, as it was marketed first.

12.2.1 Selection Criteria

12.2.1.1 Inclusion Criteria

All studies selected patients with treatment failures or contraindications to other preventatives, as suggested by the European Guidelines on the use of mAbs [7]. Indeed, this allowed to assess treatment efficacy in difficult-to-treat patients excluded by clinical trials and to reproduce a context as similar as possible to daily clinical practice. Specifically, one study selectively enrolled patients with highly resistant chronic migraine, who had previously failed at least ten pharmacological and non-pharmacological preventatives including OnabotulinumtoxinA [8]. Two studies [9, 10] required a minimum of five or more prior preventive failures plus the failure of OnabotulinumtoxinA for those diagnosed with CM, as requested by German authorities for the reimbursement of erenumab. Conversely, a British study [11] recruited patients with so-called refractory CM, who had previously unsuccessfully received or had contraindications to all preventative classes. Two Italian studies [12, 13] included patients with two or more prior preventive failures. For the remaining studies selection criteria were not available [4, 14].

Regarding the form of migraine, most studies focused on CM; however, four evaluated the efficacy of erenumab also in patients with EM reporting at least four monthly migraine days (MMDs) and multiple prior preventive treatment failures [9, 12–14]. Patients with high-frequency (i.e., 8–14 migraine days per month) EM were also enrolled by a study evaluating the efficacy of galcanezumab [6].

Concurrent preventives were allowed in most cases; in one case even onabotulinumtoxinA was permitted in association with erenumab [4]. Changes of add-on preventative were not allowed in a German study [9] to avoid the bias related to the drug switch; whereas two of the Italian studies allowed concurrent preventatives switch during the follow-up [12, 13]. The remaining authors did not specify the possibility to apply these changes during the study period.

12.2.1.2 Exclusion Criteria and Study Failure

In the considered studies, the only restrictive exclusion criterion was comorbidity with psychiatric disorders [13, 15], in accordance with the European guidelines, which suggest avoiding mAbs in patients with severe mental disorders because of their possible reduced compliance to the therapy.

Furthermore, treatment withdrawal was allowed in case of insufficient response to the drug, defined as MMDs reduction lower than 30% compared with baseline or in case of severe adverse events (SAE) and/or lack of compliance and satisfaction.

12.2.2 Dosage

Erenumab is available in two monthly dosages: 70 mg and 140 mg; dose escalation is a suitable option in case of insufficient response [16]. Only two studies assessed the efficacy of the sole 70 mg dosage [9, 14]. Another study allowed dose escalation since the second injection of the drug and the administration of erenumab 140 mg from the beginning in case of high number of previous preventative failures [13]. This indication was in line with the results of randomized trials [16] and with the LIBERTY trial inclusion criteria [17].

Regarding galcanezumab, a loading dose of 240 mg was administered at treatment start followed by 120 mg monthly courses.

12.2.3 Outcomes of the Studies

Overall, the efficacy of mAbs had been assessed in terms of reduction of migraine and headache days, responder rate, intensity of the attacks, acute medication, and triptans usage. Changes in the disability impact of disease were also evaluated through several scales, with the headache impact test (HIT-6) being used the most. Other efficacy outcomes were the resolution of medication overuse and the reversion of CM in EM. With respect to safety outcomes, the proportion and types of adverse events were considered.

12.2.3.1 Monthly Migraine, Monthly Headache Days, and Responder Rate

Given the different definitions of migraine and headache attacks in the ICHD-3 [18], monthly migraine days and monthly headache days are slightly different metrics. However, they both measure the frequency of the attacks and assess disease severity. Changes in MMDs were recorded by all the studies apart from two Italian studies [12, 15], which evaluated treatment efficacy only in terms of MHDs reduction and did not refer to the ICHD-3 for the definition of the outcome. Conversely, changes in MHDs were not evaluated by five studies [4, 6, 8, 13, 14].

An additional measure of the frequency of migraine attacks is the responder rate, which is used to determine whether to continue the therapy. The studies defined 30%, 50%, 75%, and 100% responder rates as 30%, 50%, 75%, and 100% reduction in MMDs, respectively, compared with baseline.

12.2.3.2 Monthly Acute Medication Consumption

In addition to the aforementioned metrics, the monthly symptomatic consumption also assesses disease severity. Most studies considered the monthly number of days requiring the intake of symptomatic medications (acute medication days, AMDs), others the precise number of pain medications taken in a month (monthly pain medication intake, MPMI) [6, 8, 14, 15]. All the studies, except for two [4, 15], registered changes in this outcome before and after the treatment. Furthermore, two of them separately considered monthly usage of triptans and analgesics [10, 13].

12.2.3.3 Disability, Comorbidities, and Patient-Reported Outcomes

Considering the disability burden of migraine, several scales were used to evaluate treatment-driven improvements in quality of life. The HIT-6 scale was used in the majority of the studies, while three Italian studies [6, 13, 15] evaluated disability also using the Migraine Impact and Disability Assessment Scale (MIDAS). The aforementioned studies [13, 15] further assessed the efficacy of erenumab on psychiatric symptoms, such as depression and anxiety through the Beck Depression Inventory (BDI) and Generalized Anxiety Disorder (GAD-7) Questionnaire. They both also evaluated changes in allodynia, which is a common symptom associated with migraine, through the Allodynia Symptom Checklist-12 (ASC-12) [13, 15]. One of the two researches [15] performed a comprehensive assessment of patients' status using different scales: Hamilton Depression Rating Scale (HDRS), Hamilton Anxiety Rating Scale (HARS); Medical Outcomes Study (MOS) Sleep Scale, migraine-specific quality-of-life questionnaire (MSQ), Pain Catastrophizing Scale (PCS), and MIGraine attacks-Subjective COGnitive impairments scale (MIG-SCOG).

12.2.3.4 Assessment Time Points

The efficacy outcomes were recorded at the third and sixth month from treatment start, apart from three studies [9, 10, 14], whose follow-up period was shorter, and one [12], whose follow-up was 12-month long.

12.3 Patients' Baseline Characteristics

A total of 1411 patients were enrolled in the real-life observational studies considered, mostly suffering from CM. Females ranged from 71% to 90% of the population and the mean age was between 44 and 53 years across the studies. Overall, the recruited patients suffered from severe forms of migraine with a long disease duration ranging from a mean of 5.1–33.1 years and multiple prior preventive treatment failures (from 3.23 to 13). Medication overuse affected 28.5–100% patients. Regarding the baseline characteristics of disease, the mean MMDs was between 12.5 and 26, AMDs 10.1–13.5, MPMI 14–30, and HIT-6 65.9–69.3 (Tables 12.1 and 12.2 and Fig. 12.1).

Table 12.1 Design and baseline characteristics of real-life studies

Study (year)	Type of study	Country	Type of drug and dosage	Months of follow-up	Disease duration, mean years ± SD	Sample size	Migraine forms, % of patients	Mean age ± SD	Female sex, % of patients	Medication overuse, % of patients	Mean prior treatment failures ± SD/ (IQR)	Concurrent preventatives, % of patients
Barbanti et al. (2019) [14]	Open-label	Italy	Erenumab 70 mg	2	5.13 ± 1.8 EM, 5.4 ± 2.6 CM	78	16% EM, 84% CM	47 ± 14.4 EM, 47.1 ± 10 CM	71%	61.5% EM, 84.6% CM	29.1 ± 15.3 EM, 30.2 ± 11.7 CM	61.5% EM, 66.2% CM
Raffaelli et al. (2020) [10]	Retropective open-label	Germany	Erenumab 70 and 140 mg	3	NA	139	100% CM	53.4 ± 10.2	83.5%	NA	3.6 ± 1.2 plus BoNTA	14.4%
Lambru et al. (2020) [11]	Prospective open-label	UK	Erenumab 70 and 140 mg	6	13 ± 11.9	162	100% CM	46 ± 13	83.3%	54%	8.4 ± 3.6	NA
Russo et al. (2020) [15]	Prospective open-label	Italy	Erenumab 70 and 140 mg	6	33.1 ± 1.2	70	100% CM	46.9 ± 1.4	78.6%	91.4%	4.7 ± 0.3	57%
Ornello et al. (2020) [13]	Open-label	Italy	Erenumab 70 and 140 mg	6	28.2 ± 13.3	89	5.6% EM, 94.4% CM	46.8 ± 11.2	87.6%	71.9%	3.23	41.6%
Scheffler et al. (2020) [9]	Retrospective open-label	Germany	Erenumab 70 mg	3	NA	100	26% EM, 74% CM	52.9 ± 9.1 EM 45.8 ± 12.9 CM	88.4% EM 91.2% CM	66% CM	NA	NA
Robbins et al. (2020) [4]	Retrospective open-label	US	Erenumab 70 and 140 mg	6	NA	220	100% CM	53[a]	77.7%	NA	NA	NA
Kanaan et al. (2020) [5]	Retrospective Cohort study	US	Erenumab 70 and 140 mg	7	NA	418	NA	NA	NA	NA	NA	NA

(continued)

Table 12.1 (continued)

Study (year)	Type of study	Country	Type of drug and dosage	Months of follow-up	Disease duration, mean years ± SD	Sample size	Migraine forms, % of patients	Mean age ± SD	Female sex, % of patients	Medication overuse, % of patients	Mean prior treatment failures ± SD/ (IQR)	Concurrent preventatives, % of patients
Ranieri et al. (2020) [12]	Prospective open-label	Italy	Erenumab 70 and 140 mg	12	NA	30	30% EM, 70% CM	44 ± 11.5	90%	28.5%	NA	NA
Pensato et al. (2020) [8]	Prospective open-label	Italy	Erenumab 70 and 140 mg	3	18.9 ± 10.1	39	100% CM	49.8 ± 8.3	64%	100%	13 (11–16)	64%
Vernieri et al. (2020) [6]	Prospective open-label	Italy	Galcanezumab LD 240 mg +120 mg	3	NA	66	26.9% EM, 73.1% CM	48.2 ± 10.6	85.3%	NA	NA	NA

BoNTA OnabotulinumtoxinA, *CM* Chronic migraine, *EM* Episodic migraine, *NA* Not available, *SD* Standard deviation
[a]Median value

Table 12.2 Main results of real-life studies

		Baseline			T1				T2				AEs
Study	MMDs	AMDs or MPMI	HIT-6	NRS	MMDs	AMDs or MPMI	HIT-6	NRS	MMDs	AMDs or MPMI	HIT-6	NRS	
Barbanti et al. [14]	10.9 ± 2 EM, 22 ± 6 CM	13.8 ± 4.2EM, 4.7 ± 26.1 CM**	69.4 ± 6.5 EM, 69.2 ± 6.8 CM	8 ± 1.3 EM, 8 ± 0.9 CM	7.1 ± 5 EM, 9.8 ± 7.5 CM (month 1)	7 ± 6 EM, 9.5 ± 7.3CM (month 1)**	58.3 ± 11.7 EM, 59 ± 8.7 CM (month 1)	7 ± 6 EM, 5.4 ± 1.6 CM (month 1)	0 EM, 6.6 ± 4 CM (month 2)	0 EM, 5.5 ± 0.2.8 CM (month 2)**	36 EM, 57 ± 7 .9 CM (month 2)	0 EM, 5.3 ± 0.7 CM (month 2)	7.8% (1/78)
Raffaelli et al. [10]	14.6 ± 5.3	11.9 ± 4.6	NA	NA	10.5 ± 6.4 (month 1)	7 ± 4.4 (month 1)	NA	NA	10.9 ± 6.4 (month 3)	6.5 ± 2.9 (month 3)	NA	NA	37.4% (52/139)
Lambru et al. [11]	19.7 ± 0.7	11.5 ± 0.7	67.6 ± 0.4	NA	13.7 ± 1 (month 3)	3.3 ± 0.7 (month 3)	59.9 ± 0.9 (month 3)	NA	12.2 ± 1.5 (month 6)	4 ± 3.1 (month 6)	60.1 ± 1.3 (month 6)	NA	48% (77/162)
Russo et al. [15]	21.1 ± 0.7*	25 ± 3.7**	65.9 ± 1.1	8.6 ± 0.1	11.4 ± 0.9* (month 3)	NA	60.7 ± 1.2 (month 3)	8.1 ± 0.1 (month 3)	8.9 ± 0.7* (month 6)	NA	59.5 ± 1.4 (month 6)	7.9 ± 0.1 (month 6)	25.7% (18/70)
Ornello et al. [13]	19.8 ± 8.4	13.5 ± 10.6	66	8	7.8 (month 3)	2 (month 3)	NA	NA	4 (month 6)	2 (month 6)	55.5 (month 6)	8 (month 6)	22.5% (20/89)
Scheffler et al. [9]	9.4 ± 2.9 EM 15.7 ± 6.6 CM	10.2 ± 4.7 EM, 11.6 ± 6.1 CM	NA	NA	5.9 ± 5.4 EM, 10.9 ± 7.8CM (month 3)	5.7 ± 5.1 EM, 8.9 ± 5.9 CM (month 3)	NA	NA	NA	NA	NA	NA	42% (42/100)
Kanaan et al. [5]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	70% (169/418)
Ranieri et al. [12]	19.6 ± 7.3*	10.1 ± 5.5	NA	NA	11.9 ± 8.8* (month 3)	5.9 ± 6 (month 3)	NA	NA	7.9 ± 6.9* (month 12)	4.4 ± 4.0 (month 12)	NA	NA	26.6% (8/30)
Pensato et al. [8]	26.0 (19–30)	NA	66.7 ± 8.2	6.8 ± 1.1	NA	NA	NA	NA	13.0 (9.5–27) (month 3)	NA	57.3 ± 13.8 (month 3)	6.3 ± 1.7 (month 3)	28% (11/39)
Vernieri et al. [6]	11.5 ± 4 EM, 20.0 ± 7 CM	8 ± 1 EM, 20 ± 17 CM**	64.5 ± 3 EM, 67.5 ± 7 CM	12 ± 5 EM, 8 ± 1 CM	NA	NA	NA	NA	3 ± 2 EM, 8.5 ± 10 CM (month 3)	5 ± 6 EM, 7.5 ± 6 CM** (month 3)	55.0 ± 9 EM 59.0 ± 13 CM (month 3)	6 ± 3 EM, 6.5 ± 9 CM (month 3)	NA

AEs Adverse events, *AMDs* Acute medication days, *HIT-6*, *MMDs* Monthly migraine days, *MHDs** Monthly headache days, *MPMI*** Monthly pain medication intake, *NA* Not available, *NRS* Numerical rating scale

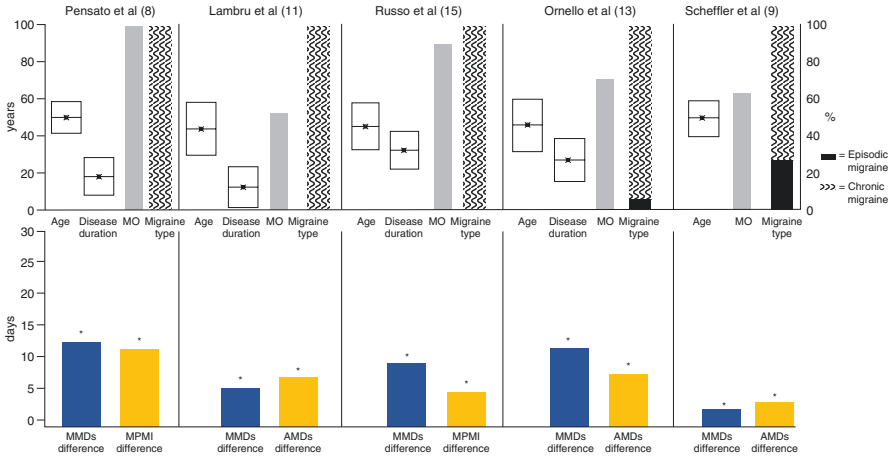

Fig. 12.1 Baseline and 3-month outcomes of the main real-life observational studies. The upper side of the figure reports baseline patients' characteristics for each study: mean age and mean years of disease duration followed by the proportion of patients with medication overuse and patients suffering from chronic or episodic migraine. At the bottom of the figure we report the difference between baseline and the third month of follow-up for monthly migraine days and acute medication days or monthly pain medication intake. Abbreviations: *AMDs* Acute medication days, *MMDs* Monthly migraine days, *MO* Medication overuse, *MPMI* Monthly pain medication intake. Asterisks indicate statistical significance

12.4 Results

Overall, the studies demonstrated that, in patients suffering from more severe forms of migraine, mAbs efficacy and safety were similar or even superior compared with clinical trials results (Table 12.2).

12.4.1 Erenumab

12.4.1.1 Efficacy

Monthly Migraine Days and Responder Rate

All the studies registered a significant reduction of mean MMDs compared with baseline. Indeed, a decrease in mean MMDs[1] between −2.5 and −13 was registered at the third month of follow-up, when MMDs ranged from 5.2 to 13.7 across

[1] These results also include difference of MHDs compared with baseline and mean MHDs at the follow-up time points of the two studies [12, 25], which measured the efficacy of erenumab only as changes in this metric. Notably, they did not specifically refer to the ICH-3 for the definition of the outcome.

studies. At the sixth month of follow-up three studies [11, 13, 15] registered a decrease in mean MMDs between −7.5 and −15 compared with baseline. Two Italian studies [8, 13] observed the greatest reduction of this outcome even if they recruited patients with higher mean number of MMDs at baseline [8, 13] and a high rate of previous treatment failures [8] (Fig. 12.1).

The 50% responder rate was between 34% and 59.1% at the third month of follow-up and between 18% and 63.3% at the sixth month; the 75% responder rate was between 13% and 70% at the third month and between 22% and 34.8% at the sixth month; whereas the 100% responder rate reported by two studies [4, 13] was 4.5% and 10% at the third month. The gradual reduction of the 50% responder rate and the increase of higher responder rates over the months might be explained by an increased responder to the drug due to dose escalation, when allowed, or to a late sensitization to the treatment (Fig. 12.2).

Acute Medications Consumption and Medication Overuse

At the third month of follow-up, the reduction of AMDs compared with baseline was between −3.6 and −8.2 [9–13]; while the sole study reporting changes in MPMI registered a reduction of −12 [8]. At the sixth month of follow-up two studies reported −8 and −7.5 AMDs, respectively [11, 13]. The drug was able to resolve medication overuse in a relevant proportion of patients ranging from 25% to 71.9% across the studies.

HIT-6 and Other Scales

In terms of disability evaluation, the mean HIT-6 score was between 57.3 and 60.7 at the third month of follow-up, and between 55.5 and 60.1 at the sixth month of follow up. In the two Italian studies [13, 15] erenumab also demonstrated to be effective in reducing mean MIDAS score, which dropped respectively from a mean baseline of 46 to 13 and from 108.1 to 51 [13, 15] at the sixth month of follow-up. Similarly, the BDI and ASC-12 significantly reduced in both studies compared with baseline values.

Erenumab seems to exert its action even on other symptoms associated with migraine and on psychiatric symptoms other than depression as demonstrated by the reduction of MSQ, HDRS, HARS, MOS sleep scale, and PCS scores at the sixth month from treatment start. MIG-SCOG score, which measures the cognitive impairment during migraine attacks, was the sole metric not registering a remarkable reduction at the sixth month of follow-up [15].

12.4.1.2 Safety

All the studies reported the adverse events (AEs) registered during the treatment. Overall, the drug proved to have a good safety profile with few severe adverse events and adverse events causing treatment withdrawal. The total proportion of

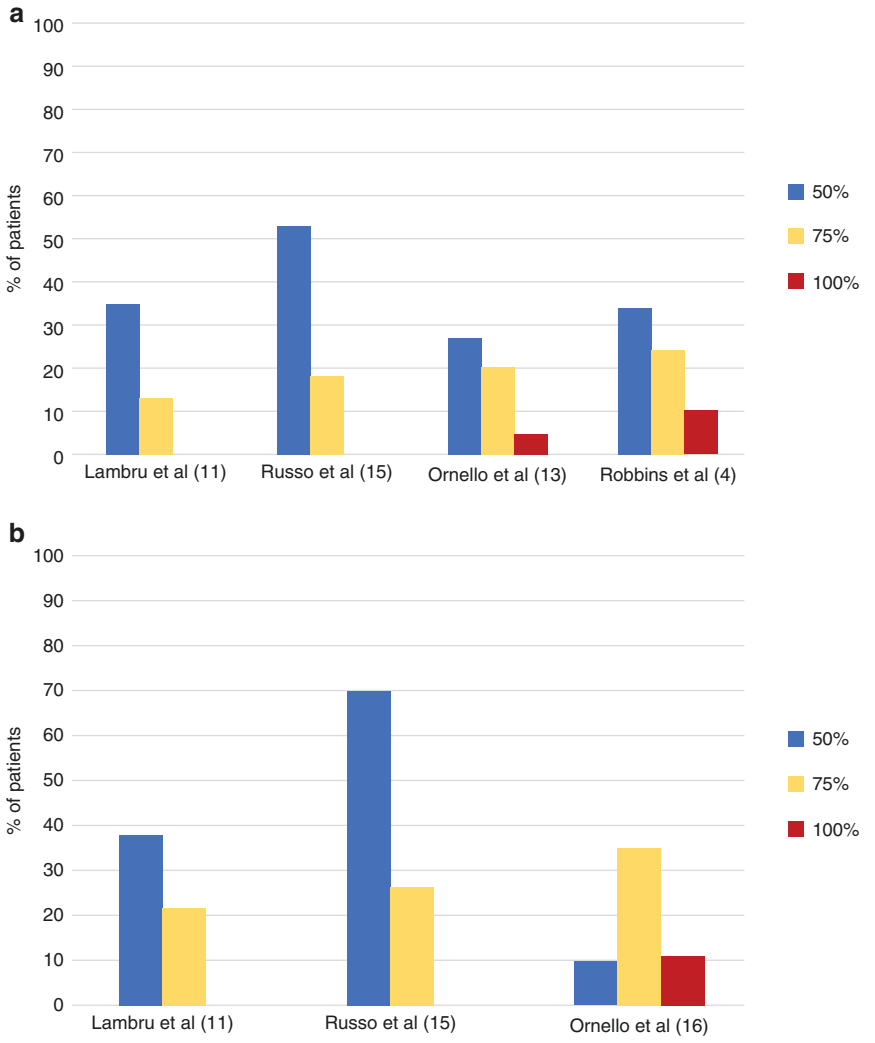

Fig. 12.2 Proportion of patients reporting a 50%, 75%, and 100% reduction of monthly migraine days compared with baseline at the third months (**a**) and sixth month of follow-up (**b**)

patients with AEs ranged from 7.8% to 70%. The most common side effect was constipation (18.7%–51%) followed by injection site pain (3.6%–24%) and respiratory infections (32%–4.3%). Notably, severe adverse events leading to treatment withdrawal rarely occurred. Examples were severe constipation, small bowel obstruction, stroke, AR relapse, severe fatigue, and severe allergy. The first Italian real-life study reported the lowest number of AEs, with only one case of injection-site erythema over 78 patients [14]. Conversely, the highest proportion of side effects was reported by the American survey [5], where 70% of patients had AEs.

This high percentage might be due to the patients' baseline characteristics and the so-called nocebo effect, which is consequence of patient's expectation of AEs. The same study registered also one death, whose connections with the drug could not be proved.

Erenumab, as well as the other mAbs, is contraindicated in case of pregnancy due to the paucity of specific safety profile data available. In the abovementioned survey, there was one unexpected pregnancy, which led to subsequent treatment withdrawal; however, the pregnancy outcome had not been reported [5].

12.4.2 Galcanezumab

12.4.2.1 Efficacy

Despite the paucity of data about galcanezumab, its efficacy in a real-life setting seems to be similar to erenumab. Indeed, the preliminary study on this drug recorded an overall −7.25 ($P < 0.0001$) decrease of MMDs compared to baseline together with a 50% responder rate of 76.6% and a 75% responder rate of 32.95% at the third month of follow-up [6]. Apart from the latter outcome, there was quite a remarkable difference between EM and CM, which might be justified by the different baseline patients' characteristics (patients with EM had 3 (2 IQR) mean baseline MMDs; while patients with CM 8.5 (10 IQR)).

Similarly, MPMI remarkably reduced in both groups with a mean reduction of respectively—3 in patients with EM and—12.5 in those with CM at the third month of follow-up.

The study also evaluated disability scores such as HIT-6 and MIDAS. Specifically, the HIT-6, decreased from 64.5 (3 IQR) to 55.0 (9 IQR) in those suffering from EM and from 59.0 (13 IQR) to 67.5 (7 IQR) in those with CM. A significant reduction was observed even in the MIDAS score, which, however, was less remarkable.

12.4.2.2 Safety

A small proportion of patients reported adverse events classified as minor side effects. Specifically, 9.8% reported AEs such as itching, dizziness at the first month of follow-up, 7.3% at the second and 2.4% at the third months.

12.5 Case Reports

Few case reports were recently published evaluating the efficacy and safety of erenumab with no information on the other mAbs.

There is evidence of vascular side effects, which might raise concerns on the safety profile of the drug. Indeed, one case of ischemic stroke has been reported in a young woman suffering from episodic migraine without aura. The cerebrovascular accident occurred 34 days after the first dose of erenumab 70 mg and few hours after the self-administration of rizatriptan for a typical migraine attack. No other causes of the ischemia had been found [19]. This is not the sole case of ischemic stroke reported so far: one was registered by one of the American studies [4] as severe AE occurring in a 21-year-old woman diagnosed with hemiplegic migraine, whose only vascular risk factor was low-dose estrogen contraception. In both cases, the drug might have not been the sole cause of the stroke; indeed, the use of a triptan as well as the hormone treatment, even if at low dosages, might have contributed due to their well-known action at vascular sites. Furthermore, three cases of Raynaud's phenomenon have been reported among the vascular drug-related AEs: only one was a new onset while the others were occasional occurrences of this phenomenon [20]. Erenumab might exert a peripheral inhibition of the CGRP-mediated vasodilation of skin vessels, thus impairing skin vascular responses.

As highlighted by clinical trials and real-world studies, the most common AEs are gastrointestinal disorders; notably, one case has been reported of paralytic ileus in a patient treated with erenumab 140 mg, who underwent a planned abdominal surgery. A clear causal relationship with the mAb was not identified, but it could have played a role due to the well-known negative effects of CGRP on gastrointestinal motility [21].

The mechanisms of action of erenumab also allow the combination with other immunomodulating therapies. Indeed, a patient with Myasthenia Gravis and chronic migraine receiving erenumab and subcutaneous Ig achieved a good control of both diseases [22]. No interference was recorded because erenumab does not act as an immunomodulatory agent: this might lead to a future combined administration with other monoclonal antibodies.

Regarding the efficacy of erenumab on other types of headache, five cases of patients suffering from both cluster headache (CH) and migraine have been reported. They all observed a reduction in the frequency and intensity of CH attacks after at least 3 months of treatment with erenumab 140 mg, suggesting that this type of headache needs a prolonged and higher dosage therapy [23].

Clinical trials have not tested the mAbs add-on gepants as treatments acting on the same pathogenetic pathway. However, this might be a suitable therapeutic strategy in difficult-to-treat patients as demonstrated by the cases of two patients, who achieved good results combining erenumab and rimegepant 75 mg. The antibody switch might be another option in these cases; indeed, three patients not responding to erenumab showed a positive response to galcanezumab [24].

Further real-life studies will identify patients, who might benefit most from mAbs alone or in combination with other CGRP-targeted treatments, such as gepants, without an increased vascular risk. For this purpose, a deep and comprehensive evaluation of patients' comorbidities and risk factors is paramount [25].

12.6 Limitations

The main limitation of these studies was the low proportion of patients with EM. Although the implementation of mAbs in clinical practice privileged patients with CM, who need effective preventatives, patients with EM do not have any migraine-specific preventatives. Moreover, high-frequency EM carries a similar burden of disability compared with CM [26] and early treatment with anti-CGRP mAbs might slow down or even prevent progression to CM [27].

Future larger studies will also need to evaluate the efficacy of mAbs in patients suffering from migraine with aura and menstrual-related migraine. Up to date, no information has been provided regarding the efficacy of these drugs on the aura phenomenon, as the low number of patients enrolled in real-life studies did not allowed to setup subgroup analyses. The effect of anti-CGRP mAbs is strictly peripheral; indeed, they do not cross the blood-brain barrier [28]; whereas aura is known as a cortical phenomenon. Therefore, anti-CGRP mAbs are expected to be effective on migraine pain, but not on the mechanisms generating aura. Despite this, the efficacy of erenumab in clinical trials suggests that patients with migraine with aura can benefit from anti-CGRP mAbs. Poor evidence is available even on menstrual-related migraine, which should be evaluated by further observational studies. So far, only a clinical trial subgroup analysis showed the efficacy of erenumab in this condition [29].

The heterogeneity of concurrent medications could have affected the outcomes of real-life studies and partially explains their better results compared with clinical trials, where other preventatives had previously been stopped. Future larger collaborative studies will likely provide stratified results, according to concurrent medication and suggest the most effective combination treatments.

12.7 Conclusions and Further Perspectives

Real-life studies demonstrated mAbs efficacy in difficult to-treat patients, who have been largely excluded by previous clinical trials. Overall, patients presented longer disease duration, multiple prior preventive failures, and higher baseline migraine severity compared with clinical trials. As mAbs proved to be effective even in cases where OnabotulinumtoxinA had previously failed [10, 13], this might suggest that mechanisms of action of the two drugs do not fully overlap and that combining them might be useful in case of treatment refractoriness. One of the American studies [4] was the sole allowing OnabotulinumtoxinA as concurrent treatment; however, it did not compare the efficacy of the two combined treatments with erenumab alone, thus underestimating the possible efficacy of the add-on drug.

The refractoriness to all preventatives, including mAbs, observed in some cases might be caused by still partially unclear mechanisms. A deep comprehension of these mechanisms will open up new therapeutic options, such as the combination of

mAbs with other preventatives acting on the CGRP pathway like gepants [30]. The slightly different actions of mAbs targeting CGRP and those specific for CGRP receptor could explain the incomplete blockade of the pathway exerted by one another and the subsequent therapeutic gain of the antibody switch. Further data are required to prove the validity of these strategies in the clinical practice, as well as provide selection criteria of the most suitable add-on therapy according to patients' characteristics.

Real-word data also demonstrated that erenumab can resolve medication overuse without a prior detoxification process. Clinical trials have already observed similar results with all types of mAbs [3]; however, future observational studies are required to assess whether detoxification strategies combined with mAbs significantly improve the results of medication overuse withdrawal.

In most studies, both the dosages of erenumab were administered; however, data did not provide clear guidance on the best timing for dose escalation. Overall, the higher dosage ensured a greater response to the treatment: the future goal would be the identification of response predictors, which could lead to tailor-made and more effective therapies.

The proportion of adverse events was sometimes higher than clinical trials possibly due to the higher rate of comorbidities or the aforementioned nocebo effect. No significant differences have been registered in the types of AEs, confirming gastrointestinal disorders and injection-site reactions as the most common side effects. However, no cerebrovascular accidents were reported by clinical trials [3, 31].

Larger multicenter studies with longer follow-up will likely contribute to the identification of predictors of response to mAbs and provide a more comprehensive assessment of the efficacy of these drugs on quality of life, headache intensity, and response to symptomatic treatments. Lastly, studies evaluating the effects of treatment suspension will be fundamental to establish the optimal therapy duration, which is currently suggested by expert consensus [7].

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Chapter 13
Migraine Versus Cluster Headache and Potential Other Indications

Matilde Capi, Luana Lionetto, Valerio De Angelis, and Paolo Martelletti

13.1 Introduction

Migraine and other headache disorders are among the most prevalent disorders worldwide. Migraine alone afflicts about 12% of the global population, attacking up to 17% of women and 6% of men each year [1]. The Global Burden of Disease Study, a large study on global health, has recently reevaluated the disability caused by headache disorders, ranking them at second place worldwide among the most disabilitating disorders in terms Years Lived with Disabilty (YLDs). Migraine alone in 2000 was ranked 19th, 3rd in 2015, and 2nd in 2017 in both men and women aged under 50 [2, 3].

Few research studies have focused on the impact of cluster headache (CH) on personal and global healthcare, disability, and quality of life, despite the excruciating pain that it provokes [4]. It is a rare condition classified as trigeminal autonomic cephalalgia and an effective management of CH requires the integration of several clinical strategies [5]. The rarity of this condition might explain the lack of research findings on its impact. The few studies published so far show that CH has a strong impact on daily lives activity, affecting patients' well-being, and social, familial, and workplace functioning. Furthermore, CH has a high healthcare costs, as well as

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A. Maassen van den Brink, P. Martelletti (eds.), *Monoclonal Antibodies in Headache*, Headache, https://doi.org/10.1007/978-3-030-69032-8_13

indirect costs due to a decrease in the working capacity and therefore results in a significant socioeconomic impact on society as a whole [6].

Post-traumatic headache (PTH) after traumatic brain injury (TBI) is classified as a secondary headache disorder because of the close temporal relation to another disorder known to cause headache [7]. TBI is a very frequent event, a major threat to global health as 69 million individuals worldwide are estimated to sustain such injury each year [8]. PTH is defined as persistent post-traumatic headache (PPTH) when the pain does not resolve within 3 months [7] and is associated with higher rates of anxiety and depression symptoms and reduced quality of life. There is no recognized pharmacological treatment for PTH, and incorrect clinical management might lead to medication overuse (MO) and the chronicization of headache.

13.2 Migraine

In recent years, numerous studies have investigated the role of CGRP receptors in the pathophysiological mechanisms of migraine. CGRP is a peptide composed of 37 amino acids, present in the α and β isoforms at sensory neurons level, in the unmyelinated C fibers and in the A-δ fibers, where it is involved in the transmission of pain and has a vasodilatory action. This receptor is related to a G protein and is made up of three subunits: receptor activity-modifying protein 1 (RAMP1), calcitonin-like receptor (CLR), and the receptor component protein (RCP). It has been shown that, during spontaneous migraine attacks, the concentration of CGRP increases at the level of the external jugular vein and how it decreases following the administration of triptans in parallel with the symptomatic improvement. Antagonizing the CGRP pathway is therefore a promising new strategy for both acute treatment and migraine prophylaxis. This implication has led to the development of new molecules called monoclonal antibodies (mAbs) available acting on the calcitonin gene-related peptide (CGRP) pathway which can be used for migraine prevention [9]. They are highly specific molecules for their target, have a long half-life, but are about 500 times larger than gepants or triptans. Their pharmacokinetic characteristics make them ideal for chronic treatment and the minimization of adverse events. At present, four mAbs have been evaluated in clinical trials for episodic and chronic migraine: eptinezumab, fremanezumab, galcanezumab for CGRP, and erenumab for CGRP canonical receptor. Eli Lilly and Company has developed Galcanezumab, a fully humanized monoclonal antibody against human calcitonin gene-related peptide (CGRP). The Food and Drug Administration (FDA) approved it on 27 September 2018. In two phase 1 and phase 2 clinical trials (EVOLVE-1, EVOLVE-2) subcutaneous galcanezumab has been tested in patients with episodic migraine. Results show a significantly reduction in the mean number of monthly migraine days (MMDs) versus placebo. In addition, during the 6-month double-blind treatment patients treated with galcanezumab reported a reduction in the number of monthly acute migraine-specific medication days (MSMDs), further supporting its efficacy for preventive therapy.

Adverse events most frequently reported were injection site pain, erythema, abdominal pain, and upper respiratory tract infections [10].

Lundbeck Seattle Biopharmaceuticals developed Eptinezumab, a fully humanized IgG1 antibody, designing it to bind specifically to both alpha and beta forms of the human calcitonin gene-related peptide (CGRP). FDA approved it in February 2020 for the preventive treatment of migraine headaches in adults. Recently, Lipton et al. reported a significant reduction in migraine incidence compared to baseline levels in patients treated with eptinezumab. Moreover, one-third of patients treated with eptinezumab 100 and 300 mg experienced a ≥75% reduction in migraine days 1 month after the initial administration, and the preventative effect of these dosages was maintained through the full 12-week dosing interval [11]. Adverse events reported were upper respiratory tract infection and urinary tract infections. Teva Pharmaceuticals USA developed Fremanezumab, a humanized monoclonal antibody targeting human calcitonin gene-related peptide (CGRP), for the prevention of migraine headaches. FDA approved it in September 2018. Phase II and two Phase III randomized clinical trials showed that subcutaneous fremanezumab is an effective treatment for episodic and chronic migraine with respect to placebo, with a significant reduction in the average number of headache days. Mild and moderate adverse events were related to the injection-site reactions relatively frequently [12].

Erenumab (AMG-334), a human monoclonal antibody, binds and antagonizes the calcitonin gene-related peptide receptor (CGRPR) and is designed for the preventative treatment of migraine. Monthly subcutaneous administration of Erenumab for a 6-month period produced a reduction of at least 50% in mean migraine days per month in a randomized, double-blind, multicenter, placebo-controlled trial in episodic migraine patients. The administration of Erenumab had no impact on the hepatic function [13].

The European Headache Federation (EHF) defined specific guidelines for the use of mAbs in the treatment of episodic and chronic migraine. Up to date, the use of mAbs seems to have an improved safety profile compared to other drugs used for the treatment of migraine. Many studies (n = 28) including phase II and phase III clinical trials have been used to define the safety and efficacy of fremanezumab and erenumab. Particularly, the guidelines recommend the use of erenumab, fremanezumab, or galcanezumab in patients with episodic migraines characterized by poor response to other treatments, or that cannot use other drugs due to adverse events and comorbidities. However, data on possible drug–drug interactions with mAbs are still lacking, and therefore further studies are still needed in order to understand possible adverse events in patients with other comorbid pathologies [9].

13.3 Cluster Headache

Cluster headache (CH) is a primary headache disorder also known as trigeminal autonomic cephalgia. This rare disorder hits the 0.1% of the population and is therefore difficult to investigate extensively. It is considered as the worst pain known to

men and therefore, despite its rarity, its recognition and treatment are vital. CH is defined as a unilateral pain with at least one autonomic symptom ipsilateral to the headache. CH attacks might occur up to eight times a day, usually at the same time of day, most often at night. These daily attacks occur from weeks to months, followed by a remission for months or years. It has been mentioned before that there is no single clear source of CH. Some data suggest that a defect in the central pathway of pain control and an autonomic nervous system dysregulation lead to a dysfunction in the supraspinal control of pain. Studies have suggested also a dysfunction in inter and intracellular signaling pathways of GABA, ion channels, and inflammation-related molecules such as IL-2, adhesion molecules, and histamine [14]. A recent clinical trial (NCT02964338) involving 259 participants investigated efficacy and safety profiles of Fremanezumab (TEV-48125) for the preventative treatment of chronic cluster headache (CCH). Unfortunately, the clinical development program of fremanezumab in cluster headache (ENFORCE Phase III) was suspended by Teva Pharmaceutical because it did not meet primary endpoints.

Galcanezumab (Emgality, Eli Lilly) has been approved by FDA as the first therapy for the treatment of episodic cluster headache in adults in September 2018. Emgality effectiveness has been proved in a clinical trial (NCT02397473) involving 106 patients in a 3-week period comparing the average number of cluster headaches per week. After the treatment, patients taking Emgality reported 8.7 fewer weekly cluster headache attacks than they did at baseline, compared to 5.2 fewer attacks for patients on placebo. Adverse events reported were related to hypersensitivity reactions in the injections site happening several days after the administration. In case of serious hypersensitivity reaction treatment should be discontinued.

European Medicines Agency (EMA) did not approve Emgality for cluster headache prophylaxis, basing this decision on the results of a single study in patients with episodic cluster headache and concluding that the results did not show a clear efficacy of Emgality in preventing attacks and therefore its benefits did not outweigh its risks. Further studies are needed to define galcanezumab although long-term safety profile, but given its efficacy and short-term tolerability profile galcanezumab might be an important option for the preventative treatment of CH.

13.4 Persistent Post-Traumatic Headache

The causes of persistent headache following concussion are poorly understood. The International Headache Society [7] classifies post-traumatic headache as a secondary headache caused by trauma or injury to the head that develops within 7 days following trauma. The classification defines acute post-traumatic headache if the pain disappears after 3 months, while persistent post-traumatic headache if the pain lasts longer. Persistent post-traumatic headache (PPTH) and migraine are phenotypically similar and differentiated only by the presence of a mild or moderate traumatic brain injury (mTBI). A great percentage of patients with post-traumatic headache (PTH) reports clinical symptoms similar to migraine including headache,

nausea, dizziness, insomnia, poor concentration, memory problems, and sensitivity to light and sound. Up to date, the pharmacological treatment of post-traumatic headache uses acute or preventive medications for primary headache disorders because PTH is phenotypically similar to migraine and tension-type headache [15]. FDA has not yet approved any pharmacological treatment for PTH and this lack of data might lead to the prescription of unnecessary therapies that might interfere with comorbidities such as depression and anxiety [16]. Another consequence of these inappropriate therapies is medication overuse, which can contribute to the chronicization of migraine in 20–30% of children and adolescents with chronic daily headache unrelated to concussion [17]. A recent open-label randomized clinical trial showed that erenumab is effective in the treatment of PPTH, reducing days with headache pain moderate or severe, suggesting it might be also useful in the preventative treatment of PTH. A low frequency of adverse events has been reported, but further studies are needed to assess the effectiveness of erenumab against placebo as well as other preventive medications [18]. It is reasonable to think that patients with migraine phenotype PPTH who do not respond to conventional therapies might try anti-CGRP monoclonal antibody treatment. In these patients, the therapy with mAbs should be carefully evaluated considering patients' features like age, pubertal state, and medical comorbidities [16]. Similar studies should be carried out in order to validate these findings and determine if mAbs could be implied in first-line treatment of PTH with migraine phenotype.

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