Characterization of Ubrogepant: A Potent and Selective Antagonist of the Human Calcitonin Gene-Related Peptide Receptor^S

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ABSTRACT

A growing body of evidence has implicated the calcitonin gene-related peptide (CGRP) receptors in migraine pathophysiology. With the approval of monoclonal antibodies targeting CGRP or the CGRP receptor, the inhibition of CGRP-mediated signaling emerged as a promising approach for preventive treatments of migraine in adults. Recently, small-molecule anti-CGRP treatments have shown efficacy for treating migraine. The current studies aimed to characterize the pharmacologic properties of ubrogepant, an orally bioavailable CGRP receptor antagonist for the acute treatment of migraine. In a series of ligand-binding assays, ubrogepant exhibited a high binding affinity for native ($K_i = 0.067$ nM) and cloned human ($K_i = 0.070$ nM) and rhesus CGRP receptors ($K_i = 0.079$ nM), with relatively lower affinities for CGRP receptors from rat, mouse, rabbit, and dog. In functional assays, ubrogepant potently blocked human α -CGRP-stimulated cAMP response (IC_{50} of 0.08 nM) and exhibited highly selective antagonist activity for the CGRP receptor compared with other members of the human calcitonin receptor family. Furthermore, the in vivo CGRP

receptor antagonist activity of ubrogepant was evaluated in a pharmacodynamic model of capsaicin-induced dermal vasodilation (CIDV) in rhesus monkeys and humans. Results demonstrated that ubrogepant produced concentration-dependent inhibition of CIDV with a mean EC_{50} of 3.2 and 2.6 nM in rhesus monkeys and humans, respectively. Brain penetration studies with ubrogepant in monkeys showed a cerebrospinal fluid:plasma ratio of 0.03 and low CGRP receptor occupancy. In summary, ubrogepant is a competitive antagonist with high affinity, potency, and selectivity for the human CGRP receptor.

SIGNIFICANCE STATEMENT

Ubrogepant is a potent, selective, orally delivered, small-molecule competitive antagonist of the human CGRP. In vivo studies using a pharmacodynamic model of CIDV in rhesus monkeys and humans demonstrated that ubrogepant produced concentration-dependent inhibition of CIDV, indicating a predictable pharmacokinetic-pharmacodynamic relationship.

Introduction

Migraine is a highly prevalent, chronic neurologic disease and the leading cause of disability in people aged 15–49 years (Burch et al., 2018; Steiner et al., 2018). Commonly used acute

treatments for migraine attacks include triptans, opioids, nonsteroidal anti-inflammatory drugs, ergotamine derivatives, barbiturates, and combination analgesics (Holland et al., 2013; Martelletti, 2017). However, the utility of these treatments is limited by low levels of adherence and patient satisfaction stemming largely from inadequate efficacy and poor tolerability (Holland et al., 2013; Lipton et al., 2013; Messali et al., 2014; Serrano et al., 2015; Martelletti, 2017). As a result, many people with migraine discontinue acute treatments and may experience uncontrolled attacks or migraine disease progression (Holland et al., 2013; May and Schulte, 2016; Thorlund et al., 2016).

Theories explaining the pathophysiology of migraine have shifted away from a purely vascular disease model toward a neurogenic theory focusing on the neuropeptide calcitonin gene-related peptide (CGRP) (Humphrey, 2007; Eftekhari

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ABBREVIATIONS: AM, adrenomedullin; AMY, amylin; cAMP, cyclic adenosine monophosphate; CGRP, calcitonin gene-related peptide; CIDV, capsaicin-induced dermal vasodilation; CLR, calcitonin receptor-like receptor; CNS, central nervous system; CSF, cerebrospinal fluid; CT, calcitonin; CTR, calcitonin receptor; HEK293, human embryonic kidney-derived; hERG, human ether-a-go-go-related gene; PD, pharmacodynamic; PET, positron emission tomography; PK, pharmacokinetic; RAMP, receptor activity modifying protein.

and Edvinsson, 2010; Moore and Salvatore, 2012; Gonzalez-Hernandez et al., 2018). CGRP is a 37-amino acid peptide and potent vasodilator present at elevated levels in the trigemino-vascular system during migraine attacks (Goadsby et al., 1990; Russell et al., 2014). The calcitonin family of peptides includes calcitonin (CT), amylin (AMY), adrenomedullin (AM), and CGRP (Poyner et al., 2002). The CGRP receptor is made up of a CT receptor (CTR)-like receptor (CLR) and receptor activity modifying protein 1 (RAMP1) (Eftekhar and Edvinsson, 2010; Kiriya and Nochi, 2018). Distribution of CLR and RAMP1 has been mapped to the cytoplasm of trigeminal neurons, at peripheral sites on the intracranial vasculature (in the smooth muscle cells), in the dura matter (both vascular and avascular localization), and in the brainstem (Edvinsson and Warfvinge, 2019). The AMY receptors are a complex of the CTR and RAMP1 (i.e., AMY₁), RAMP2 (i.e., AMY₂), or RAMP3 (i.e., AMY₃) that have a high affinity for AMY. The AMY₁ receptor also binds CGRP, is found in the trigeminal ganglion, and has been implicated in animal models of pain signaling; however, its role in migraine pathophysiology remains to be determined (Gebre-Medhin et al., 1998; Poyner et al., 2002; Walker et al., 2015; Kiriya and Nochi, 2018; Edvinsson and Warfvinge, 2019). The AM receptors are a complex of the CLR and RAMP2 (AM₁) and RAMP3 (AM₂), which have a high affinity for AM (Kiriya and Nochi, 2018). The function of AM receptors in migraine is unknown, and, unlike with CGRP, intravenous infusion of AM was not found to precipitate migraine pain (Petersen et al., 2009).

A substantial amount of scientific data implicates the CGRP pathway in the underlying physiologic mechanisms associated with migraine (Goadsby et al., 1990; Lassen et al., 2002; Edvinsson, 2015). Furthermore, previously investigated small-molecule CGRP receptor antagonists have demonstrated efficacy in the treatment of migraine, although development was eventually halted because of safety concerns (Hewitt et al., 2011; Ho et al., 2014; Hargreaves and Olesen, 2019). The clinical success of monoclonal antibodies targeting CGRP also provides support for the CGRP receptors as a promising therapeutic target for migraine (Dodick, 2019).

Ubrogepant is an orally bioavailable, potent, and specific CGRP receptor antagonist that was approved in December 2019 for the acute treatment of migraine in adults with or without aura. Ubrogepant is chemically distinct from previous small-molecule CGRP receptor antagonists (Ubrely, 2019) (Yasuda et al., 2017). In clinical trials, ubrogepant provided substantial pain relief and return to function, and was generally well tolerated. The objective of the present communication is to describe and characterize the pharmacologic profile of ubrogepant.

Materials and Methods

In Vitro Pharmacology

Binding Affinity. To assess ubrogepant's affinity and selectivity for CGRP and AM receptors, cloned CGRP and AM₂ receptors were stably expressed in human embryonic kidney-derived (HEK293) cells. To assess ubrogepant's affinity for AMY₁ receptors, cloned AMY₁ receptors were transiently expressed in monkey kidney-derived cells by transfection with equal amounts of CTR and RAMP1 to monitor selectivity of ubrogepant for this receptor.

For receptor binding assays, membrane fractions were isolated from cell homogenates from the HEK293 or monkey kidney-derived

cells or homogenates of cerebellum isolated from rhesus, rat, mouse, rabbit, and dog. Human [¹²⁵I]CGRP and [¹²⁵I]AM and rat [¹²⁵I]AMY were used as radioligands for the binding assays. Nonspecific binding was determined by 10 μ M MK-3207 (Salvatore et al., 2010), a structurally distinct CGRP receptor antagonist. The tests were conducted in 1 ml of binding buffer (10 mM HEPES, pH 7.4, 5 mM MgCl₂, and 0.2% bovine serum albumin) for 3 hours at room temperature containing 10 pM human [¹²⁵I]CGRP, 10 pM human [¹²⁵I]AM, or 40 pM rat [¹²⁵I]AMY in the presence of several concentrations of ubrogepant. The assays were terminated by filtration through 0.5% polyethyleneimine-treated GF/B glass fiber filter plates with ice-cold wash buffer (10 mM HEPES, pH 7.4 and 5 mM MgCl₂). Scintillation fluid was added to the plates and radioactivity quantitated using a Packard TopCount NXT scintillation counter (PerkinElmer, Shelton, CT). Dose-response curves were plotted to determine half-maximal inhibitory concentration (IC₅₀) values and converted to K_i values using the equation $K_i = IC_{50}/(1 + ([\text{ligand}]/K_d))$. Data are presented descriptively using group means and S.E.M.s, unless otherwise noted.

Functional Potency. The effect of ubrogepant on CGRP-, AM-, or CT-induced increases in cyclic adenosine monophosphate (cAMP) was assessed in HEK293 cells expressing human CGRP receptors, rhesus CGRP receptors, cloned human AM₁ (CLR/RAMP2), AM₂ (CLR/RAMP3), cloned human AMY₁ (CTR/RAMP1) or AMY₃ (CTR/RAMP3), or human CTR alone.

Cells were pre-incubated at 2000 cells/well in 384-well plates with various concentrations of ubrogepant for 30 minutes at 37°C. In the human CGRP receptor functional assays, potency was assessed with and without 50% human or rhesus serum. The cyclic nucleotide inhibitor isobutyl-methylxanthine was added to the cells at a concentration of 300 μ M for 30 minutes at 37°C followed by stimulation with 1.0 nM human α -CGRP (human and rhesus CGRP receptor assays), 1.0 nM human AM (human AM₁ and AM₂ receptor assays), 0.5 nM rat AMY (cloned human AMY₁ and AMY₃ receptor assays), or 0.2 nM human CT (human CTR assay) for 20 minutes at 37°C. After agonist stimulation, cAMP concentration was measured with the homogeneous time-resolved fluorescence cAMP Dynamic Assay (Cisbio, Bedford, MA).

Dose-response curves were plotted and IC₅₀ values determined from a four-parameter logistic fit as defined by the equation $y = [(a - d)/(1 + x/c)^b] + d$, where y = response, x = dose, a = maximum response, d = minimum response, c = inflection point, and b = slope. For the CGRP assays, Schild analysis was used as a measure of competitive antagonism by plotting log (DR-1) versus log [B], where DR is the ratio of α -CGRP half-maximal effective concentration (EC₅₀) values in the presence and absence of ubrogepant and [B] is the antagonist concentration. The X-intercept is equal to the pA₂ and the K_B calculated using the formula $pA_2 = -\log K_B$.

Specificity/Off-Target Profiling. The specificity of ubrogepant was assessed in ligand binding or functional assays across 116 targets (Supplemental Table 1; Olon Ricerca Bioscience, Concord, OH) and against the human ether-a-go-go-related gene (hERG), which encodes the inward-rectifying voltage-gated potassium channel in the heart and is involved in cardiac repolarization. Ubrogepant was tested at a concentration of 10 μ M in conventional radioligand binding and enzyme assays, and a concentration dose-response curve was generated when significant activity was observed.

The hERG ligand-binding assay was conducted using membrane fractions isolated from HEK293 cells stably expressing cloned hERG. [³⁵S] MK-499 (Wang et al., 2003) was used as the radioligand and 10 μ M astemizole (Suessbrich et al., 1996) was used to determine nonspecific binding. Binding assays were conducted in 1 ml of binding buffer (60 mM KCl, 71.5 mM NaCl, 1 mM CaCl₂, 2 mM MgCl₂, and 10 mM HEPES, pH 7.4) containing 25 pM [³⁵S] hERG ligand in the presence of several concentrations of ubrogepant for 3 hours at room temperature. The assay was terminated by filtration through 0.05% polyethyleneimine-treated GF/B glass fiber plates with ice-cold wash buffer (10 mM HEPES, pH 7.4). Plates were dried under vacuum at 37°C for 1 hour, scintillation fluid was added, and radioactivity quantitated using a Packard TopCount NXT scintillation counter.

Binding Kinetics. Saturation binding assays were performed by combining increasing concentrations of [3 H]-ubrogepant, 10 μ M CGRP receptor antagonist MK-3207 for nonspecific binding, and 50 μ g SK-N-MC membranes per well. The mixtures were incubated overnight (18 hours) at room temperature in binding buffer (10 mM HEPES, pH 7.4, 5 mM MgCl₂, and 0.2% bovine serum albumin) in a total volume of 1 mL.

Association kinetic assays were performed by combining 40 pM [3 H]-ubrogepant with 50 μ g SK-N-MC membranes per well in binding buffer and incubating at room temperature for various times (1–90 minutes). Dissociation kinetic assays were performed by combining 40 pM [3 H]-ubrogepant with 50 μ g SK-N-MC membranes per well in binding buffer and incubating at room temperature for 3 hours. At that point, 10 μ M of the CGRP receptor antagonist MK-3207 was added and dissociation was monitored for various intervals (1–300 minutes). All assays were terminated by filtration through 0.5% polyethyleneimine-treated GF/B glass fiber plates with ice-cold wash buffer (10 mM HEPES, pH 7.4, and 5 mM MgCl₂). Plates were dried under vacuum at 37°C for 1 hour, scintillation fluid was added, and radioactivity quantitated using a Packard TopCount NXT scintillation counter.

In Vivo Pharmacology: Assessment of Pharmacodynamic Effect

The pharmacodynamic (PD) activity of CGRP receptor antagonists in vivo has been established and validated using the capsaicin-induced dermal vasodilatation (CIDV) model in both rhesus monkeys (Salvatore et al., 2008, 2010) and human clinical trials (Li et al., 2015). Therefore, the in vivo PD activity of ubrogepant was assessed using CIDV assay in rhesus monkeys and humans in this study. The protocol for the human CIDV study was reviewed and approved by the Independent Ethics Committee of the University Hospitals of Leuven, Belgium. Before enrollment, all participants gave informed consent in writing after a full verbal and written explanation of the study. The study was conducted in accordance with local law, the ethical principles of the Declaration of Helsinki, and Good Clinical Practice guidelines.

Rhesus Monkey CIDV. Twenty-one adult rhesus monkeys (≤ 10 per individual study) were used across six studies to determine the effect of vehicle and active test agent on CIDV. Animals were provided at least 5–7 procedure-free days between studies. For the CIDV test, animals were maintained on isoflurane anesthesia, and four O-rings (8 mm internal diameter) were placed on the ventral forearm. After equilibration, the baseline response 20 minutes after application of 2 mg capsaicin (dissolved in 30% ethanol, 30% Tween20, and 40% water) in one ring was measured using a laser Doppler imager (Moor Instruments, Wilmington, DE).

Next, three successive intravenous bolus + intravenous infusions of vehicle or one to three rising doses of ubrogepant were administered. Five minutes after the start of each infusion, 2 mg capsaicin was applied to one of the remaining rings. Scans were completed for each ring before the start of each infusion and 20 minutes after capsaicin application (i.e., 25 minutes after start of infusion). Study A targeted ubrogepant plasma levels of 0.5, 5, and 50 nM in five male and two female rhesus monkeys; Study B targeted plasma levels of 5, 50, and 150 nM in six male and one female rhesus monkeys; Study C targeted plasma levels of 1, 5, and 10 nM in three female rhesus monkeys; Study D targeted plasma levels of 150, 500, and 500 nM in seven male rhesus monkeys; Study E targeted plasma levels of 1, 10, and 10 nM in two male and two female rhesus monkeys; and Study F targeted plasma levels of 400 nM in 10 male rhesus monkeys. Dosages were calibrated to achieve specified target plasma levels and to provide adequate coverage of the dynamic range of the pharmacokinetic (PK)/PD curve.

Blood samples to determine plasma ubrogepant concentrations, response curves, and inhibitory concentrations were obtained at 20 minutes after application during each test period. An empirical maximal effect ($E_{\max}$) model was used to describe the PK/PD

relationship of ubrogepant for inhibition of CIDV in rhesus monkeys. Blood flow was described as a baseline blood flow plus an incremental blood flow as a result of CIDV and blockade of CIDV by ubrogepant through an $E_{\max}$ relationship. The model was represented as $F = F_0 + F_{\text{caps}} \cdot [1 - E_{\max} \cdot C/(EC_{50} + C)]$, where F is the observed blood flow (mean perfusion values) measured by laser Doppler imaging, F_0 the baseline blood flow, F_{caps} the incremental blood flow due to application of capsaicin, $E_{\max}$ the maximal percentage inhibition of ubrogepant, C the plasma concentration of ubrogepant, and EC_{50} the plasma concentration of ubrogepant corresponding to 50% inhibition of CIDV. Data were pooled across six rhesus CIDV studies (intravenous dose range from 0.06 to 100 μ g/kg) (see Supplemental Table 2 for doses for each study). Interindividual variability parameters were selected using forward substitution (significance level of 0.05) for F_0 , F_{caps} , $E_{\max}$, and EC_{50} . Covariate assessments focused on looking for study-to-study differences in F_0 and response to capsaicin (F_{caps}). Model fitting was performed using NONMEM VII (ICON plc, Dublin, Ireland) using first-order conditional estimation with interaction.

Human CIDV. Healthy young males aged 18–50 years were administered oral ubrogepant during a randomized, double-blind, placebo-controlled, four-period crossover study (EudraCT Number: 2011-002359-32). Participants were required to fast for 8 hours before their ubrogepant dosing and pretreatment procedures. Inhibition of CIDV was measured by laser Doppler scan at 1 and 5 hours after a single oral dose of ubrogepant (0.5 mg, 5 mg, and 40 mg). The doses were selected to capture the expected dynamic range of exposure-response curve based on the estimated EC_{50} of 3.2 nM from rhesus CIDV experiments. Doppler scans were also conducted before the study (for inclusion purposes) and before study drug administration (predose). Capsaicin was applied 30 minutes before each postdose laser Doppler scan at 0.5 and 4.5 hours after study drug administration. Capsaicin was applied as single topical doses of 300 μ g/20 μ l and 1000 μ g/20 μ l capsaicin in 10-mm rubber O-rings at two sites on the volar surface of the participant's left and right forearms. The data were used to determine the concentration of drug necessary to achieve the EC_{90} using PK/PD modeling and using two doses of capsaicin, with an approach similar to the primate studies outlined above.

Brain Penetration Studies

CGRP Receptor Occupancy by Ubrogepant in Rhesus Monkey Brain by Positron Emission Tomography. All animal studies were conducted in accord with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 2011) and were approved by the Institutional Animal Care and Use Committee at Merck & Co., Inc. (West Point, PA). The quantification of CGRP receptor occupancy by ubrogepant was conducted in four anesthetized adult male rhesus monkeys by positron emission tomography (PET), using the PET tracer for the CGRP receptor [11 C]MK-4232 (Hostetler et al., 2013). A baseline PET scan was performed with [11 C]MK-4232 in the absence of ubrogepant. To establish steady plasma levels of study drug, an intravenous bolus plus constant infusion of ubrogepant

Fig. 1. Chemical structure of ubrogepant.

TABLE 1

Mean (S.E.M.) binding affinities (K_i , nM) of ubrogapant for human and nonhuman CGRP receptors^a

Species	Human Cloned	Human SK-N-MC	Rhesus	Rat	Mouse	Rabbit	Dog
Binding affinity	0.07 ± 0.006	0.067 ± 0.004	0.079 ± 0.005	9.6 ± 1.1	11.6 ± 1.1	11.0 ± 0.5	47.0 ± 4.0

SK-N-MC, human neuroblastoma cell line.

^a $n = 3-19$.

was started 60 minutes before intravenous bolus injection of ~5 mCi [¹¹C]MK-4232 and continuing for the duration of the scan. PET studies were acquired for 120 minutes after [¹¹C]MK-4232 administration.

Plasma concentrations of [¹¹C]MK-4232 for each study were obtained from the measurement of total radioactivity in arterial plasma, with correction for the fraction of intact tracer as determined by high-performance liquid chromatography, and plasma levels of ubrogapant were determined from arterial blood samples. Tissue time-activity curves were fit, and receptor occupancy was calculated using the Lassen plot. For each receptor-occupancy PET study, the estimated receptor occupancy was associated with the average plasma drug levels during the PET scan.

Cerebrospinal Fluid Penetration of Ubrogapant in Rhesus Monkeys. The cerebrospinal fluid (CSF):plasma ratio of ubrogapant was assessed in three adult male rhesus monkeys with chronically implanted cisterna magna catheters and port systems for repeated noninvasive collection of CSF. Additional details related to CSF collection have been previously published (Gilberto et al., 2003; Salvatore et al., 2010). After oral gavage of ubrogapant at 10 mg/kg, CSF and plasma samples were collected at baseline and 0.5, 1, 2, 4, 8, and 24 hours and analyzed for compound levels.

Results

Ubrogapant is an orally bioavailable CGRP receptor antagonist developed for the acute treatment of migraine. The chemical structure of ubrogapant is presented in Fig. 1.

Receptor Binding and Functional Potency of Ubrogapant. Ubrogapant was a potent inhibitor of [¹²⁵I]CGRP binding to the cloned and native human CGRP receptors with a mean K_i (±S.E.M.) of 0.07 ± 0.006 nM and 0.067 ± 0.004 nM, respectively (Table 1). Comparable affinity for ubrogapant was observed with the rhesus CGRP receptor 0.079 ± 0.005 nM; however, markedly lower affinity was found for rat, mouse, rabbit, and dog receptors (K_i > 9.5 nM). Affinity for the human AM₂ receptor was significantly lower (K_i = 2059 ± 122 nM) than that for the human CGRP receptor, but ubrogapant did display moderate affinity for the recombinant human AMY₁ receptor by inhibiting [¹²⁵I]-rAMY binding with a K_i of 8.2 nM (individual K_i = 6.5, 9.8).

Ubrogapant potentially blocked human α-CGRP-stimulated cAMP responses with a mean (±S.E.M.) IC₅₀ of 0.081 nM (0.005 nM) in human CGRP receptor-expressing HEK293 cells and 0.07 nM (0.02 nM) in rhesus CGRP receptor-expressing HEK293 cells (Table 2). The addition of 50% human or 50% rhesus serum reduced the apparent potency of ubrogapant by approximately 2.4- and 4.0-fold for human (0.19 ± 0.01 nM) and rhesus (0.30 ± 0.01 nM) CGRP receptors, respectively. Using Schild regression, ubrogapant caused potent, dose-dependent rightward shifts (data not shown) in the agonist dose-response curves, K_B = 0.017 nM, with no reduction in the maximal agonist response.

Specificity and Selectivity of Ubrogapant. In a specificity assessment against 116 enzyme, receptor, and ion channel binding assays (Supplemental Table 1), ubrogapant

showed weak affinity for the dopamine transporter (K_i = 4440 nM), which is significantly lower than its affinity for the CGRP receptor. Saturation binding studies using [³H]-ubrogapant demonstrated that specific binding was saturable to SK-N-MC membranes, with a K_D of 0.041 nM.

Ubrogapant displayed no significant antagonism of AM-induced cAMP stimulation of the human AM₁ or the human CTR at concentrations greater than 20,000 nM, whereas potency was somewhat greater at the AM₂ receptor and consistent with binding data for that receptor (Table 3). Similarly, in blockade of AMY-stimulated cAMP responses, ubrogapant demonstrated antagonist activity on the human AMY₁ and AMY₃ receptors at potencies comparable to its affinity for those receptors based on [¹²⁵I]-rAMY binding (Table 3).

Pharmacodynamic Assessment. Dermal vasodilation response to capsaicin was found to be concentration- and time-dependent. Application of vehicle alone did not significantly inhibit resulting blood flow increases. The PK/PD relationship for inhibition of CIDV by ubrogapant was estimated based on data from six rhesus CIDV studies (Study A-F, with intravenous doses ranging from 0.06 to 100 μg/kg) using a population E_{max} model (Supplemental Table 2). Studies E (0.3 and 3 μg/kg) and F (50 μg/kg) were found to be significant covariates for baseline blood flow before administration of capsaicin or ubrogapant (i.e., F₀) (Fig. 2). Ubrogapant has a mean EC₅₀ of 3.19 nM (S.E.M., 3.65 nM; Supplemental Table 3), corresponding to an estimated EC₉₀ of 29 nM. The E_{max} for inhibition of CIDV by ubrogapant is 0.732 (±0.0859).

In the human PD study of CIDV, a dose-dependent decrease was observed with a single dose of ubrogapant compared with placebo at 1 and 5 hours postdose, regardless of capsaicin concentration used (Table 4). The estimated EC₅₀ and EC₉₀ values for ubrogapant for inhibition of CIDV in humans were 2.56 and 23 nM, respectively.

Brain Penetration. In assessments of CGRP receptor occupancy in adult male rhesus monkey brain by PET, ubrogapant achieved receptor occupancy (0%–16%) at plasma levels of 53–203 nM, which are higher than the rhesus monkey CIDV EC₉₀ of 29 nM. Tests of central nervous system (CNS) penetration of ubrogapant in cisterna magna-ported conscious adult male rhesus monkeys found a CSF-to-plasma concentration ratio of 0.03 (Table 5).

TABLE 2

Mean (S.E.M.) functional potency (IC₅₀, nM) of ubrogapant for cloned CGRP receptors in HEK293 cells^a

Cloned Human CGRP Receptor		Cloned Rhesus CGRP Receptor	
No Serum	+ 50% Human Serum	No Serum	+ 50% Rhesus Serum
0.081 ± 0.005	0.19 ± 0.01	0.07 ± 0.02	0.30 ± 0.01

IC₅₀, half-maximal inhibitory concentration.^a $n = 3-21$.

TABLE 3
Selectivity of ubrogepant for cloned CTR, AM₁, AM₂, AMY₁, and AMY₃ receptors in HEK293 cells^a

Target Receptor	Selectivity Potency Difference Over CGRP Receptor ^b (IC ₅₀ = 0.08 nM)
AM ₁	>10,000
AM ₂	>10,000
Calcitonin (CTR)	>10,000
AMY ₁	105
AMY ₃	2737

IC₅₀, half-maximal inhibitory concentration.
^an = 3–19.
^bCalculated as (target receptor IC₅₀ – CGRP receptor IC₅₀)/CGRP receptor IC₅₀.

Discussion

Ubrogepant is a potent CGRP receptor antagonist developed for use in the acute treatment of migraine. In the present study, ubrogepant exhibited a high affinity for the human CGRP receptor (*K_i*, 0.07 nM). Additionally, ubrogepant exhibited species specificity, showing high affinity for the human and rhesus CGRP receptor and reduced affinity for other nonhuman receptors.

Ubrogepant displayed high selectivity for the human CGRP receptor versus the human AM₁, AM₂, CT, and AMY₃ receptors but selectivity was reduced against the AMY₁ receptor. This observation is consistent with the RAMP1-dependence of other small-molecule CGRP receptor antagonists (Moore and Salvatore, 2012; Walker et al., 2015). Incubation of HEK293 cells expressing the human CGRP receptor with ubrogepant blocked the α-CGRP–stimulated cAMP response, with an IC₅₀ of 0.08 nM. Increasing concentrations of ubrogepant caused parallel rightward shifts in the α-CGRP dose-response curves in the cAMP functional assay, and the dose-ratio plot displayed a straight line. Additionally, screenings of 116 off targets showed

that ubrogepant was highly selective for the CGRP receptor, with weak binding affinity for dopamine transporter (*K_i* of 4440 nM). This dopamine activity is likely to be pharmacologically irrelevant at the plasma concentrations projected shown to be efficacious doses in humans.

In the CIDV model in the rhesus monkey, capsaicin activates vanilloid receptor 1, producing neurogenic inflammation and vasodilation via activation of dorsal root reflexes and the release of vasoactive mediators, which is driven primarily by CGRP. This response can be blocked by CGRP receptor antagonists, thus permitting the assessment of ubrogepant potency in vivo against endogenously released CGRP (Dux et al., 2003; Hershey et al., 2005). Based on the PK/PD relationship for inhibition of CIDV by ubrogepant, the estimated mean EC₅₀ and EC₉₀ values were 3.19 and 29 nM, respectively. Population PK/PD CIDV modeling in the present rhesus in vivo study showed that the E_{max} for inhibition of CIDV by ubrogepant is 0.732, suggesting that CGRP is the primary, but not the only, contributor to CIDV (substance-P and histamine may also play a role in vasodilation), which is generally consistent with results from other CIDV studies for CGRP compounds (Vu et al., 2017). Similar results were observed in human studies using the CIDV model and resulted in similar estimates for mean EC₅₀ and EC₉₀ values of 2.56 and 23 nM, respectively. Taken together, these rapid assessments of CGRP receptor antagonism activity in non-human primates and human participants indicate a predictable PK-PD relationship for ubrogepant across species.

In the CNS penetration study, the CSF:plasma ratio was 0.03. Limited penetration into the CNS suggests that ubrogepant does not readily cross the blood-brain barrier, which was supported by the present receptor occupancy data that showed low central CGRP receptor occupancy (0%–16%) at plasma levels of 53–203 nM. Although the exact site of action of CGRP receptor antagonists is not known, the limited penetration of ubrogepant

Fig. 2. Ubrogepant dose-dependent inhibition of capsaicin-induced dermal vasodilation in the rhesus forearm: population model-predicted vs. observed blood flow after 2 mg capsaicin application at different plasma concentrations of ubrogepant. Data pooled from six rhesus CIDV studies (Studies A to F with intravenous dose range from 0.06 to 100 μg/kg) represented by symbols. Solid lines represent model-predicted population mean values. Studies E and F were found to have statistically significant differences in the baseline blood flow (i.e., before administration of capsaicin or ubrogepant) and thus the model-predicted population means are shown separately.

TABLE 4

Summary of capsaicin-induced dermal blood flow (mean perfusion) after single oral administration of ubrogepant or placebo with capsaicin 300 or 1000 $\mu\text{g}/20\text{ }\mu\text{l}$

Root mean square error on log scale from model: 0.384 at 1 h and 300 $\mu\text{g}/20\text{ }\mu\text{l}$, 0.290 at 5 h and 300 $\mu\text{g}/20\text{ }\mu\text{l}$, 0.345 at 1 h and 1000 $\mu\text{g}/20\text{ }\mu\text{l}$, 0.284 at 5 h and 1000 $\mu\text{g}/20\text{ }\mu\text{l}$.

Capsaicin Conc. ^b ($\mu\text{g}/20\text{ }\mu\text{l}$)	Treatment	N	Percentage Inhibition (90% CI) ^a	
			1 h	5 h
300	Placebo	16		
	0.5 mg	16	22.6 (2.7, 38.4)	15.5 (-0.8, 29.1)
	5 mg	14	53.4 (40.8, 63.3)	65.2 (58.3, 70.9)
	40 mg	16	63.4 (54.0, 70.9)	65.1 (58.4, 70.8)
1000	Placebo	16		
	0.5 mg	16	27.1 (9.5, 41.3)	3.1 (-14.7, 18.2)
	5 mg	14	60.8 (50.7, 68.7)	60.7 (53.3, 67.0)
	40 mg	16	64.9 (56.5, 71.7)	63.9 (57.3, 69.5)

^aPercentage inhibition of dermal blood flow calculated as $(1 - \text{GMR}) \times 100$ percent. Root mean square error on log scale from model: 0.384 at 1 h and 300 $\mu\text{g}/20\text{ }\mu\text{l}$, 0.290 at 5 h and 300 $\mu\text{g}/20\text{ }\mu\text{l}$, 0.345 at 1 h and 1000 $\mu\text{g}/20\text{ }\mu\text{l}$, 0.284 at 5 h and 1000 $\mu\text{g}/20\text{ }\mu\text{l}$.

^bCapsaicin was applied 0.5 and 4.5 h after administration of ubrogepant.

to the CNS is consistent with the concept that during a neurovascular headache, sensitization and activation of the trigemino-vascular system results in perivascular release of neuropeptides such as CGRP (Goadsby et al., 1988; Ho et al., 2010). The trigeminal ganglion is located outside of the blood-brain barrier and thus can be readily impacted by CGRP-focused treatments (Eftekhar et al., 2015). The limited CNS activity of ubrogepant may be beneficial in avoiding potential side effects of central CGRP antagonism, and the potential clinical benefit of this limited CNS activity is unknown.

In addition to high affinity for the CGRP receptor, ubrogepant displays affinity for the AMY_1 receptor and, to a lesser degree, the AMY_3 receptor. The 100-fold difference in ubrogepant potency between the CGRP receptor and AMY_1 receptor could be representative of the difference in α -CGRP binding between the two receptors, suggesting that ubrogepant may have a binding site similar to that of CGRP. The identification of CGRP-responsive AMY_1 receptors in the trigeminal ganglia neurons and the expression of CTR and RAMP1 proteins in the spinal trigeminal complex suggests a role for these receptors in the central processing of CGRP signaling (Walker et al., 2015). Furthermore, animal knockout studies have identified a pro-nociceptive role for AMY_1 (Gebre-Medhin et al., 1998). However, the role of the AMY_1 receptor in migraine remains largely unknown.

Inhibition of the CGRP receptor has emerged as a promising target for the acute and preventive treatment of migraine (Edvinsson, 2018). Previously investigated small-molecule CGRP antagonists have demonstrated efficacy in the treatment of migraine; however, clinical studies involving telcagepant and MK-3207 revealed potential concerns regarding drug-induced elevation of liver enzymes, and clinical development of these compounds was discontinued (Hewitt et al., 2011; Ho et al., 2014; Hargreaves and Olesen, 2019). Although the exact mechanism of this hepatotoxicity is unknown, it was hypothesized to be partly attributable to the formation of reactive metabolites and not specific to CGRP receptor antagonism (Hargreaves and Olesen, 2019). The absence of any ubrogepant-associated hepatotoxicity has been supported by safety data from recent clinical studies (Goadsby et al., 2019; Hutchinson et al., 2019).

Monoclonal antibodies to CGRP and the CGRP receptor have demonstrated efficacy in people with migraine; however, these

TABLE 5

In vivo central nervous system penetration properties of ubrogepant

	10 mg/kg	
	C_{max} (nM)	t_{max} (h)
CSF	40.9 ± 21.2	4
Plasma	1345.4 ± 537.8	4
CSF/plasma ratio	0.03	—

C_{max} , maximum plasma drug concentration; t_{max} , time to C_{max} .

medications are injectable medications approved for the preventive treatment of migraine (Tepper et al., 2017; Ajovy [package insert] 2018; Aimovig [package insert] 2018; Emgality [package insert] 2018; 2018a,b,c; Dodick et al., 2018; Stauffer et al., 2018). Efficacy for preventive treatment is generally measured at 1-month intervals and thus treatment options for migraine attacks are still needed for rapid relief (Tepper et al., 2017; Ajovy [package insert] 2018; Aimovig [package insert] 2018; Emgality [package insert] 2018; 2018a,b,c; Dodick et al., 2018b; Stauffer et al., 2018). Ubrogepant is an oral CGRP receptor antagonist approved for the acute treatment of migraine that is capable of providing freedom from pain at 2 hours (Dodick et al., 2018a; Lipton et al., 2018; Voss et al., 2016). Acute treatments are a mainstay of migraine attack management and may complement preventive treatments in some patients based on factors such as headache frequency, acute treatment response, and migraine-related disability (Goadsby and Sprenger, 2010). Furthermore, the oral route of administration for ubrogepant may be preferred by patients who require multiple acute treatments for migraine attacks, compared with the injection or infusion route of administration that is offered for sumatriptan and dihydroergotamine (O'Quinn et al., 1999, D.H.E. 45 [package insert] 2002.). Ubrogepant thus represents a new class of medication for the acute treatment of migraine attacks.

In conclusion, ubrogepant is a potent, selective, orally delivered, small-molecule competitive inhibitor of the human CGRP receptor that shows a predictable PK-PD relationship and limited penetration across the blood-brain barrier at clinically effective exposures.

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References

- Aimovig. Package insert. Amgen Inc., and Novartis Pharmaceuticals Corporation, Thousand Oaks, CA, and East Hanover, NJ.
- Ajovy. Package insert. Teva Pharmaceuticals USA, Inc., North Wales, PA.
- Burch R, Rizzoli P, and Loder E (2018) The prevalence and impact of migraine and severe headache in the United States: figures and trends from government health studies. *Headache* **58**:496–505.
- Dodick DW (2019) CGRP ligand and receptor monoclonal antibodies for migraine prevention: evidence review and clinical implications [published correction appears in *Cephalalgia* (2019) 39:1069]. *Cephalalgia* **39**:445–458.
- Dodick DW, Silberstein SD, Bigal ME, Yeung PP, Goadsby PJ, Blankenbiller T, Grozinski-Wolf M, Yang R, Ma Y, and Aycardi E (2018b) Effect of fremanezumab compared with placebo for prevention of episodic migraine: a randomized clinical trial. *JAMA* **319**:1999–2008.
- Dux M, Sántha P, and Jancsó G (2003) Capsaicin-sensitive neurogenic sensory vasodilatation in the dura mater of the rat. *J Physiol* **552**:859–867.
- Edvinsson L (2015) The journey to establish CGRP as a migraine target: a retrospective view. *Headache* **55**:1249–1255.
- Edvinsson L (2018) The CGRP pathway in migraine as a viable target for therapies. *Headache* **58** (Suppl 1):33–47.
- Edvinsson L and Warfvinge K (2019) Recognizing the role of CGRP and CGRP receptors in migraine and its treatment. *Cephalalgia* **39**:366–373.
- Eftekhari S and Edvinsson L (2010) Possible sites of action of the new calcitonin gene-related peptide receptor antagonists. *Ther Adv Neurol Disord* **3**:369–378.
- Eftekhari S, Salvatore CA, Johansson S, Chen TB, Zeng Z, and Edvinsson L (2015) Localization of CGRP, CGRP receptor, PACAP and glutamate in trigeminal ganglion. Relation to the blood-brain barrier. *Brain Res* **1600**:93–109.
- Gebre-Medhin S, Mulder H, Zhang Y, Sundler F, and Bethsholtz C (1998) Reduced nociceptive behavior in islet amyloid polypeptide (amylin) knockout mice. *Brain Res Mol Brain Res* **63**:180–183.
- Gilberto DB, Zeoli AH, Szczepa PJ, Gehret JR, Holahan MA, Sitko GR, Johnson CA, Cook JJ, and Motzel SL (2003) An alternative method of chronic cerebrospinal fluid collection via the cisterna magna in conscious rhesus monkeys. *Contemp Top Lab Anim Sci* **42**:53–59.
- Goadsby PJ, Edvinsson L, and Ekman R (1988) Release of vasoactive peptides in the extracerebral circulation of humans and the cat during activation of the trigemino-vascular system. *Ann Neurol* **23**:193–196.
- Goadsby PJ, Edvinsson L, and Ekman R (1990) Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. *Ann Neurol* **28**:183–187.
- Goadsby PJ and Sprenger T (2010) Current practice and future directions in the prevention and acute management of migraine. *Lancet Neurol* **9**:285–298.
- Goadsby PJ, Tepper SJ, Watkins PB, Ayele G, Miceli R, Butler M, Severt L, Finnegan M, Szegedi A, Trugman JM, et al. (2019) Safety and tolerability of ubrogepant following intermittent, high-frequency dosing: randomized, placebo-controlled trial in healthy adults. *Cephalalgia* **39**:1753–1761.
- González-Hernández A, Marichal-Cancino BA, MaassenVanDenBrink A, and Villalón CM (2018) Side effects associated with current and prospective anti-migraine pharmacotherapies. *Expert Opin Drug Metab Toxicol* **14**:25–41.
- Hargreaves R and Olesen J (2019) Calcitonin gene-related peptide modulators - the history and renaissance of a new migraine drug class. *Headache* **59**:951–970.
- Hershey JC, Corcoran HA, Baskin EP, Salvatore CA, Mosser S, Williams TM, Koblan KS, Hargreaves RJ, and Kane SA (2005) Investigation of the species selectivity of a nonpeptide CGRP receptor antagonist using a novel pharmacodynamic assay. *Regul Pept* **127**:71–77.
- Hewitt DJ, Aurora SK, Dodick DW, Goadsby PJ, Ge YJ, Bachman R, Taraborelli D, Fan X, Assaid C, Lines C, et al. (2011) Randomized controlled trial of the CGRP receptor antagonist MK-3207 in the acute treatment of migraine. *Cephalalgia* **31**:712–722.
- Ho TW, Connor KM, Zhang Y, Pearlman E, Koppenhaver J, Fan X, Lines C, Edvinsson L, Goadsby PJ, and Michelson D (2014) Randomized controlled trial of the CGRP receptor antagonist telcagepant for migraine prevention. *Neurology* **83**:958–966.
- Ho TW, Edvinsson L, and Goadsby PJ (2010) CGRP and its receptors provide new insights into migraine pathophysiology. *Nat Rev Neurol* **6**:573–582.
- Holland S, Fanning KM, Serrano D, Buse DC, Reed ML, and Lipton RB (2013) Rates and reasons for discontinuation of triptans and opioids in episodic migraine: results from the American Migraine Prevalence and Prevention (AMPP) study. *J Neurol Sci* **326**:10–17.
- Hostetler ED, Joshi AD, Sanabria-Bohórquez S, Fan H, Zeng Z, Purcell M, Gantert L, Riffel K, Williams M, O'Malley S, et al. (2013) In vivo quantification of calcitonin gene-related peptide receptor occupancy by telcagepant in rhesus monkey and human brain using the positron emission tomography tracer [¹¹C]MK-4232. *J Pharmacol Exp Ther* **347**.
- Humphrey PP (2007) The discovery of a new drug class for the acute treatment of migraine. *Headache* **47** (Suppl 1):S10–S19.
- Hutchinson S, Dodick DW, Treppendahl C, Bennett NL, Yu S, Guo H, and Trugman JM (2019) Ubrogepant for the acute treatment of migraine: pooled safety and tolerability from ACHIEVE I and ACHIEVE II phase 3 studies. *Headache* **59** (Suppl 1):120.
- Kiriya Y and Nochi H (2018) Role and cytotoxicity of amylin and protection of pancreatic islet β -cells from amylin cytotoxicity. *Cells* **7**.
- Lassen LH, Haderslev PA, Jacobsen VB, Iversen HK, Sperling B, and Olesen J (2002) CGRP may play a causative role in migraine. *Cephalalgia* **22**:54–61.
- Li CC, Vermeersch S, Denney WS, Kennedy WP, Palcza J, Gipson A, Han TH, Blanchard R, De Lepeleire I, Depré M, et al. (2015) Characterizing the PK/PD relationship for inhibition of capsaicin-induced dermal vasodilatation by MK-3207, an oral calcitonin gene related peptide receptor antagonist. *Br J Clin Pharmacol* **79**:831–837.
- Lipton RB, Buse DC, Serrano D, Holland S, and Reed ML (2013) Examination of unmet treatment needs among persons with episodic migraine: results of the American Migraine Prevalence and Prevention (AMPP) Study. *Headache* **53**:1300–1311.
- Lipton RB, Dodick DW, Ailani J, Lu K, Lakkis H, Finnegan M, Szegedi A, and Trugman JM (2018) Efficacy, safety, and tolerability of ubrogepant for the acute treatment of migraine: results from a single attack phase III study, ACHIEVE II. *Headache* **58**:1315–1316.
- Lipton RB, Dodick DW, Ailani J, Lu K, Finnegan M, Szegedi A, and Trugman JM (2019) Effect of Ubrogepant vs Placebo on Pain and the Most Bothersome Associated Symptom in the Acute Treatment of Migraine: The ACHIEVE II Randomized Clinical Trial. *JAMA* **322** (19), doi: 10.1001/jama.2019.16711 31742631.
- Martelletti P (2017) Acute treatment of migraine: quo vadis? *Expert Opin Pharmacother* **18**:1035–1037.
- May A and Schulte LH (2016) Chronic migraine: risk factors, mechanisms and treatment. *Nat Rev Neurol* **12**:455–464.
- Messali AJ, Yang M, Gillard P, Tsai K, Tepper SJ, Bloudek LM, and Kori SH (2014) Treatment persistence and switching in triptan users: a systematic literature review. *Headache* **54**:1120–1130.
- Moore EL and Salvatore CA (2012) Targeting a family B GPCR/RAMP receptor complex: CGRP receptor antagonists and migraine. *Br J Pharmacol* **166**:66–78.
- O'Quinn S, Davis RL, Gutterman DL, Pait GD, and Fox AW (1999) Prospective large-scale study of the tolerability of subcutaneous sumatriptan injection for acute treatment of migraine. *Cephalalgia* **19**:223–231; discussion 200.
- Petersen KA, Birk S, Kitamura K, and Olesen J (2009) Effect of adrenomedullin on the cerebral circulation: relevance to primary headache disorders. *Cephalalgia* **29**:23–30.
- Poyner DR, Sexton PM, Marshall I, Smith DM, Quirion R, Born W, Muff R, Fischer JA, and Ford SM (2002) International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacol Rev* **54**:233–246.
- Russell FA, King R, Smillie SJ, Kodji X, and Brain SD (2014) Calcitonin gene-related peptide: physiology and pathophysiology. *Physiol Rev* **94**:1099–1142.
- Salvatore CA, Hershey JC, Corcoran HA, Fay JF, Johnston VK, Moore EL, Mosser SD, Burgey CS, Paone DV, Shaw AW, et al. (2008) Pharmacological characterization of MK-0974 [N-((3R,6S)-6-(2,3-difluorophenyl)-2-oxo-1-(2,2,2-trifluoroethyl)azepan-3-yl)-4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin-1-yl)piperidine-1-carboxamide], a potent and orally active calcitonin gene-related peptide receptor antagonist for the treatment of migraine. *J Pharmacol Exp Ther* **324**:416–421.
- Salvatore CA, Moore EL, Calamari A, Cook JJ, Michener MS, O'Malley S, Miller PJ, Sur C, Williams DL Jr., Zeng Z, et al. (2010) Pharmacological properties of MK-3207, a potent and orally active calcitonin gene-related peptide receptor antagonist. *J Pharmacol Exp Ther* **333**:152–160.
- Serrano D, Buse DC, Manack Adams A, Reed ML, and Lipton RB (2015) Acute treatment optimization in episodic and chronic migraine: results of the American Migraine Prevalence and Prevention (AMPP) Study. *Headache* **55**:502–518.
- Stauffer VL, Dodick DW, Zhang Q, Carter JN, Ailani J, and Conley RR (2018) Evaluation of galcanezumab for the prevention of episodic migraine: the EVOLVE-1 randomized clinical trial [published correction appears in *JAMA Neurol* (2019) 76:872]. *JAMA Neurol* **75**:1080–1088.
- Steiner TJ, Stovner LJ, Vos T, Jensen R, and Katsarava Z (2018) Migraine is first cause of disability in under 50s: will health politicians now take notice? *J Headache Pain* **19**:17.
- Suessbrich H, Waldegger S, Lang F, and Busch AE (1996) Blockade of HERG channels expressed in *Xenopus* oocytes by the histamine receptor antagonists terfenadine and astemizole. *FEBS Lett* **385**:77–80.
- Tepper S, Ashina M, Reuter U, Brandes JL, Dolezil D, Silberstein S, Winner P, Leonardi D, Mikol D, and Lenz R (2017) Safety and efficacy of erenumab for preventive treatment of chronic migraine: a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Neurol* **16**:425–434.
- Thorlund K, Sun-Edelstein C, Druyts E, Kanters S, Ebrahim S, Bhambri R, Ramos E, Mills EJ, Lanteri-Minet M, and Tepper S (2016) Risk of medication overuse headache across classes of treatments for acute migraine. *J Headache Pain* **17**:107.
- Ubrelyv. Package insert. Allergan plc, Madison, NJ. (2019).
- Voss T, Lipton RB, Dodick DW, Dupre N, Ge YJ, Bachman R, Assaid C, Aurora SK, and Michelson D (2016) A phase IIb randomized, double-blind, placebo-controlled trial of ubrogepant for the acute treatment of migraine. *Cephalalgia* **36**:887–898.
- Vu T, Ma P, Chen JS, de Hoon J, Van Hecken A, Yan L, Wu LS, Hamilton L, and Vargas G (2017) Pharmacokinetic-pharmacodynamic relationship of erenumab (AMG 334) and capsaicin-induced dermal blood flow in healthy and migraine subjects. *Pharm Res* **34**:1784–1795.
- Walker CS, Eftekhari S, Bower RL, Wilderman A, Insel PA, Edvinsson L, Waldvogel HJ, Jamaluddin MA, Russo AF, and Hay DL (2015) A second trigeminal CGRP receptor: function and expression of the AMY1 receptor. *Ann Clin Transl Neurol* **2**.
- Wang J, Della Penna K, Wang H, Karczewski J, Connolly TM, Koblan KS, Bennett PB, and Salata JJ (2003) Functional and pharmacological properties of canine ERG potassium channels. *Am J Physiol Heart Circ Physiol* **284**:H256–H267.
- Yasuda N, Cleator E, Kosjek B, Yin J, Xiang B, Chen F, Kuo S, Belyk K, Mullens PR, Goodyear A, et al. (2017) Practical asymmetric synthesis of a calcitonin gene-related peptide (CGRP) receptor antagonist ubrogepant. *Org Process Res Dev* **21**:1851–1858.
- D.H.E. 45. Dihydroergotamine mesylate injection, solution [package insert]. Valeant Pharmaceuticals North America, Costa Mesa, CA. (2002)
- Emgality. Package insert. Eli Lilly and Company, Indianapolis, IN. (2018)

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