Relationship of the Calcitonin Gene-Related Peptide Monoclonal Antibody Galcanezumab Pharmacokinetics and Capsaicin-Induced Dermal Blood Flow in Healthy Subjects

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Abstract

Galcanezumab, a humanized monoclonal antibody targeting calcitonin gene-related peptide, was recently approved for migraine prophylaxis. The pharmacokinetic/pharmacodynamic (PK/PD) relationship between galcanezumab concentration and inhibition of capsaicin-induced dermal blood flow (CIDBF) was evaluated using first-in-human data following 6 single subcutaneous dose levels (1 to 600 mg) or multiple (4) 150 mg doses q2wk in 7 cohorts (n = 7 active/2 placebo-treated healthy subjects). Galcanezumab pharmacokinetics were best described by a 1-compartment model with delayed first-order absorption/linear elimination. Apparent estimates (between-subject variability) of clearance, volume of distribution, absorption rate constant, and lag time were 0.0106 L/h (27 %CV), 11.2 L (21 %CV), 0.0192 1/h (89 %CV), and 0.202 h, respectively. Estimated elimination half-life was about 30 days. An effect compartment link model described the concentration-effect relationship; estimated maximum inhibitory effect was 70.5% and 50% maximum inhibitory effect concentration (IC_{50}) was 1060 ng/mL. Galcanezumab showed dose- and concentration-dependent potent and durable inhibition of CIDBF. Simulated effect compartment concentrations were maintained above IC_{50} after 12 weeks of dosing. Near maximal CIDBF inhibition occurred with 150 mg biweekly for 12 weeks, lasting ≥ 24 weeks or with ≥ 30 mg q2wk or 195 mg q13wk. Quantitative modeling of galcanezumab PK/PD supported dose selection for the phase 2 proof-of-concept study.

Introduction

Calcitonin gene-related peptide (CGRP) is a promising drug target for the acute and preventative treatment of migraine and is found throughout the trigeminovascular system and in central brain regions regarded as important in migraine pathogenesis.[1] Considerable experimental and clinical findings support the role of CGRP in migraine: increases in jugular venous blood concentration of CGRP have been observed in spontaneous migraine attacks; intravenous infusion of CGRP in susceptible subjects has triggered migraine attacks; and triptan administration has resulted in reversal of elevated CGRP concentrations, an effect that corresponds with migraine symptom relief.[1,2] Importantly, double-blind, randomized placebo-controlled clinical trials have shown several small-molecule CGRP receptor antagonists to be effective in acutely treating migraine attacks,[3-5] however, off-target hepatotoxicity and formulation issues initially hampered the development of some of the first generation of these compounds.[2,6,7] A second generation of these "gepants" that has not shown this hepatotoxicity has been approved for use in treating migraine.[8,9]

For several reasons, development of monoclonal antibodies (mAbs) targeting the CGRP pathway was an alternative option to avoid many of these issues. The potential for off-target hepatotoxicity should be reduced for mAbs with non-hepatic elimination pathways based on their inherent target specificity.[10] In comparison to small molecules, the relatively longer half-life typically exhibited by mAbs makes this option amenable for prophylaxis of migraine.[11,12] Four mAbs that implicate CGRP are approved for clinical use.[13-16] Galcanezumab (LY2951742; Eli Lilly and Company), eptinezumab (ALD403; H. Lundbeck A/S), and fremanezumab (LBR-101; Teva Pharmaceutical Industries) bind to CGRP, whereas erenumab (AMG334; Amgen) selectively blocks the CGRP receptor.[12,17]

Galcanezumab is a humanized monoclonal antibody that potently binds to CGRP, with an *in vitro* half maximal inhibitory concentration of 30 pM.[1,18] In a randomized, double-blind, placebo-controlled phase 2a proof-of-concept study, galcanezumab 150 mg given as a subcutaneous injection every 2 weeks (q2wk) for 12 weeks produced a significant reduction in the mean number of monthly migraine headache days compared to placebo.[1] In a randomized multi-center phase 2b clinical trial of 410 patients with episodic migraine, galcanezumab subcutaneous injections of 120 or 300 mg were found to be safe and well tolerated and also demonstrated efficacy in reducing migraine headache days compared to placebo for the preventive treatment of migraine.[19] Subsequently, 2 large multi-center, randomized, placebo-controlled phase 3 clinical trials, EVOLVE-1 (NCT02614183) and EVOLVE-2 (NCT02614196), showed that galcanezumab 120 mg and 240 mg given every 4 weeks was significantly ($p<0.001$) superior to placebo in reducing numbers of monthly migraine headache days in patients with episodic migraine.[20,21] The randomized clinical trial REGAIN (NCT02614261) showed that galcanezumab 120 mg and 240 mg every 4 weeks was significantly ($p<0.001$) superior to placebo in reducing the numbers of monthly migraine headache days in patients with chronic migraine.[22] Together, these studies show that galcanezumab is efficacious for the prevention of migraine, safe, and well-tolerated.[20-22]

A novel pharmacodynamic assay to measure CGRP receptor antagonist activity non-invasively *in vivo* was first established in the rhesus monkey, and later extended to humans as a reproducible model that can be easily incorporated in early clinical development studies.[23,24] In this model, changes in dermal blood flow (DBF) in the forearm are measured via laser Doppler perfusion imaging (LDI) following the topical application of a capsaicin solution.[24] Capsaicin activates the transient receptor potential vanilloid type 1 receptor (TRPV1), producing

neurogenic inflammation and vasodilation via the local release of vasoactive mediators, such as CGRP, and possibly by the activation of dorsal root reflexes.[24] The resulting vasodilation in the forearm is driven primarily by CGRP and can be significantly blocked by CGRP receptor antagonists, thus permitting the assessment of antagonist potency *in vivo* against endogenously released CGRP.[25] In non-clinical testing with rats and non-human primates, galcanezumab has demonstrated inhibition of capsaicin-induced DBF increase.[18] Recent publications have described the application of capsaicin-induced change in DBF as a pharmacodynamic marker to explore the relationship between plasma drug concentration and inhibition of capsaicin-induced elevation in DBF in healthy subjects as well as migraine patients.[26,27]

Our study establishes a quantitative population pharmacokinetic/pharmacodynamic (PK/PD) model for galcanezumab to characterize the relationship between galcanezumab concentration and changes in capsaicin-induced DBF (as measured by LDI) based on the first-in-human phase I study of galcanezumab in healthy subjects. Previously published analyses for this study utilized non-compartmental pharmacokinetic (PK) analysis in relation to pharmacodynamic (PD) responses.[28] The model-based approach described herein allowed for simulation of dosing regimens with varying doses, dosing frequency, and treatment duration to guide dose selection for a subsequent clinical study of galcanezumab.

Materials and Methods

Study Design and Population

Data were obtained from a phase 1, single-site, double-blind, placebo-controlled, single-dose escalation study of the safety, tolerability, and PK of galcanezumab in healthy subjects. The study was conducted in 2 sequential parts: a single-ascending-dose (SAD) phase of 6 cohorts followed by 1 multiple-dose cohort (NCT01337596).

The study population included healthy white males, 18 to 55 years old, in 7 cohorts of 9 subjects each (7 subjects received galcanezumab and 2 received placebo) for the single- and multiple-dose assessments.

The study was conducted at the Center for Clinical Pharmacology in the university Hospital Gasthuisberg in Leuven, Belgium in accordance with the principles of the Declaration of Helsinki and was approved by the Human Investigational Review Board of the study center (that is, the Ethics Committee in Research at the university Hospital of Leuven, Belgium). Voluntary signed informed consent was obtained from each subject.

Dose Administration

Subjects in the single-dose cohorts received galcanezumab (or placebo) via subcutaneous injection(s) of 1, 5, 25, 75, 200, or 600 mg. The 600-fold range of doses required 1 of 3 injection concentrations (10, 25, or 100 mg/mL) and 1, 2, 3, or 4 injections to administer. The multiple-dose cohort received a subcutaneous injection of 150 mg galcanezumab (or placebo) on days 1, 15, 29, and 43, that is, q2wk for a total of 4 doses administered over 6 weeks.

Pharmacokinetic Sampling Strategy

For single-dose cohorts, blood samples for measurement of serum galcanezumab concentrations were collected before dosing and at 0.5, 1, 2, 4, 8, 12, and 24 hours after dosing on day 1. A single PK sample was taken on days 3, 5, 8, 14 (±2 days), 28 (±2 days), 42 (±2 days), 56 (±2 days), and 84 (±2 days). For the multiple-dose cohort, blood samples for measurement of serum galcanezumab concentrations were collected before and 24 hours after each dose. Pharmacokinetic samples were also collected on days 4, 8 (±1 day), 46, 50 (±2 days), 57 (±2 days), 71 (±2 days), 85 (±2 days), 99 (±3 days), 113 (±3 days), 141 (±3 days), and 176.

Bioanalytical Methods

Concentrations of galcanezumab were assayed using a validated enzyme-linked immunosorbent assay (ELISA) method.[29] The lower limit of quantitation of the assay method was 0.75 ng/mL. A small number of concentrations below the limit of quantitation (2.6% of samples from subjects receiving active treatment) were excluded from the dataset used for modeling.

Pharmacodynamic Endpoints and Sampling Strategies

The effect of capsaicin on DBF was tested by giving subjects dermal challenges of 1000 µg in 20 µL of capsaicin solution or vehicle. The DBF was measured using LDI. After placement of 3 rubber O-rings on the volar surface of the subject's forearm, a laser Doppler perfusion imager (HR-LDPI system, Periscan PIMII; Perimed, Sweden) was used to obtain a baseline measurement of DBF in the areas defined by the rings. Capsaicin solution was then placed in 2 of the rings and vehicle in the third ring; DBF was again measured 30 minutes later. The differences between the measurements obtained 30 minutes after the capsaicin or vehicle challenge and the baseline measurements were calculated as the change from the pre-capsaicin

baseline in DBF. The change-from-baseline measurements from the 2 capsaicin rings were averaged for use in PK/PD evaluations (capsaicin-induced DBF change).[18,23,25,30]

The dermal capsaicin challenge was performed prior to dosing as part of the screening procedures to exclude non-responders and to establish a predose (baseline) LDI evaluation.

For all single-dose cohorts, the dermal capsaicin challenge was performed during the screening period, at 48 to 56 hours after dosing on day 3, and on day 14 (±2 days), day 28 (±2 days), and day 42 (±2 days).

For the multiple-dose cohort, the dermal capsaicin challenge was performed during the screening period, and on days 14, 28, 42, 57, 71, 99 (±2 days), 113, 141, and 176, or during the visit closest in time to the days that LDI was planned.

Population Pharmacokinetic/Pharmacodynamic Analysis Methodology

Nonlinear mixed-effects modeling was performed using the computer program NONMEM® Version 7.1.2 and KIWI Version 1.1.[31,32] NONMEM analyses were performed using the first-order conditional estimation method on an Intel cluster with the Linux operating system.

A sequential approach was utilized whereby the PK model was initially developed and evaluated prior to the inclusion of the PD data. Both the PK and the PK/PD models were developed based on data from the single-dose cohorts first, then later refined with the full datasets. As supported by the exploratory graphical evaluation, a linear 1-compartment model with first-order lagged absorption and first-order elimination was used to describe the galcanezumab concentration-time data. Between-subject variability (BSV) was estimated on the absorption rate constant (k_a), apparent clearance (CL/F), and apparent volume of distribution (V/F) using exponential models.

Galcanezumab concentration data were logarithmically transformed and residual variability (RV) was estimated using a constant variance error model on the log scale.

The adequacy of the final PK model was evaluated using a simulation-based (prediction-corrected) visual predictive check (VPC, pcVPC) method.[33,34] The final model was used to simulate 1000 replicates of the analysis dataset with NONMEM.

Based on exploratory graphical analysis of galcanezumab concentrations and DBF response, an effect compartment link model was selected to characterize the PK/PD relationship between galcanezumab concentrations and the effect on DBF as measured via LDI (Figure 1). Pharmacokinetic input into this model was via individual empiric Bayesian estimates of PK parameters obtained from the final PK model for galcanezumab. The model includes the estimation of the baseline effect of capsaicin-induced DBF (time 30 minutes to time 0), an effect of vehicle control on the change from baseline in DBF, a first-order transfer rate constant for drug between the plasma and effect compartments (k_{e0}) term, reflective of the time delay between galcanezumab concentration in the serum (blood) compartment and the biophase or effect compartment at steady-state when these compartments are in equilibrium, a placebo effect, and the maximum inhibitory effect (I_{max}) and IC_{50} terms, reflecting the pharmacological effect of galcanezumab via maximum fractional inhibition of the capsaicin-induced DBF response and the effect compartment concentration associated with achieving 50% of this maximum response. Between-subject variability was estimated for the baseline capsaicin and vehicle terms, k_{e0}, I_{max}, and IC_{50}, using either additive or exponential error models. Separate additive error models for capsaicin-induced DBF and vehicle-induced DBF responses were used to estimate RV.

The equation describing the final PK/PD model for galcanezumab is provided below as Equation 1.

$$Response_{ij} = E_{veh_i} + Base_{caps_i} \times \left(1 - \left[\left(E_{max_i} \times Ce_{ij}\right)/\left(EC_{50_i} + Ce_{ij}\right)\right]\right) + E_{plc_i} \times PB_{flag_{ij}} \quad \textbf{(1)}$$

Where:

$Response_{ij}$ is the predicted change from baseline in DBF in the *i*th subject at the *j*th time;

E_{veh_i} is the predicted change from baseline in DBF following vehicle administration in the *i*th subject;

$Base_{caps_i}$ is the predicted change from baseline in capsaicin-induced DBF prior to galcanezumab administration in the *i*th subject;

E_{max_i} is the maximum reduction in the change from baseline in capsaicin-induced DBF in the *i*th subject;

EC_{50_i} is the galcanezumab concentration associated with 50% of the maximum reduction in the capsaicin-induced DBF in the *i*th subject;

Ce_{ij} is the predicted galcanezumab concentration in the effect compartment in the *i*th subject at the *j*th time;

E_{plc_i} is the predicted effect of placebo galcanezumab administration on the change from baseline in DBF response in the *i*th subject; and

$PB_{flag_{ij}}$ is an indicator variable with a value of 1 for all measurements obtained post-baseline in the *i*th subject, and 0 otherwise.

Model-Based Simulations

Simulations of DBF response were performed based on the final PK/PD model (including BSV and RV) to evaluate and compare a variety of different dosing regimen scenarios (that is, various combinations of dose amount, frequency, and duration). Dermal blood flow responses were

simulated for 1000 virtual subjects who received galcanezumab for 12 to 13 weeks with follow-up through 1 year.

In addition, observed differences from placebo in reduction of migraine headache days in patients given galcanezumab 5, 50, 120, and 300 mg subcutaneously every 28 days in a phase 2b study were graphically compared to the change from baseline in model-predicted inhibition of capsaicin-induced DBF for galcanezumab administered once monthly in doses ranging up to 300 mg.[19,35]

Results

Population Pharmacokinetic Modeling Results

A total of 746 galcanezumab concentrations collected from 49 subjects were utilized for PK analysis. Of these, 126 galcanezumab concentrations were obtained from the 7 subjects enrolled in the multiple-dose cohort. Diagnostic plots indicated a generally good fit of the PK model to the data, with some underprediction of concentrations in the 150-mg multiple-dose cohort (see Figure S1). Other alternative models were evaluated, however, due to the limited model improvement and the small sample size of the study, the linear 1-compartment model, with estimated elimination half-life of approximately 30 days, was considered an adequate and parsimonious representation of the data.

The VPC (Figure 2) illustrates the appropriateness of the PK model, with only a slight trend to underpredict drug concentrations in the 1-mg single-dose and 150-mg multiple-dose groups. In all other dose groups, the median of the simulated data generally tracks well and approximately bisects the observed data as expected.

Population Pharmacokinetic/Pharmacodynamic Modeling Results

The final analysis dataset consisted of 369 measurements of capsaicin-induced DBF and 358 measurements of change in DBF following vehicle administration collected from 63 subjects. Laser Doppler images showing capsaicin-induced increases in DBF and attenuation by galcanezumab are illustrated in Figure 3.

Mean (SE) profiles of the capsaicin-induced DBF for each dose group are provided in Figure 4. This plot illustrates the dose-response relationship for galcanezumab, where a single dose of 1 mg is similar to placebo, single doses of 5 or 25 mg achieve a slightly better response, and single doses of at least 75 mg differentiate from the other single doses and are associated with the greatest DBF response, but also exhibit substantial overlap in the mean responses. The 150-mg multiple-dose cohort appears to achieve a sustained response in the change from baseline in DBF to approximately 100 days (8 weeks after the last galcanezumab dose) before starting to return toward baseline levels.

Mean dose group and individual PK and PD profiles illustrate a consistent delay between the peak galcanezumab concentration and the nadir of the DBF response, thus supporting the use of the effect compartment link model to characterize these data (see Figure S2). Figure 5 presents an illustration of the relationship between PK and PD (excluding subjects receiving placebo and DBF measurements obtained prior to galcanezumab administration). These plots provide supporting evidence for an exposure-response relationship for capsaicin-induced DBF with galcanezumab, where increasing galcanezumab concentrations are associated with greater inhibition of capsaicin-induced increases in DBF.

The final PK/PD model parameter estimates are presented in Table 1. All PK parameters were estimated with reasonable precision (relative standard error expressed as a percentage

[%RSE] ≤ 35%), with the exception of the BSV for V/F, which was estimated with less precision (68%). Given the small sample size relevant to the estimation of several of the PD parameters (eg, estimates of the placebo effect were based on 14 subjects who received placebo compared to 49 who received galcanezumab), precision of most of the estimates was reasonable, with the exception of the BSV on I_{max}, which was estimated with relatively poor precision (357 %RSE). Diagnostic plots illustrating the fit of the PK/PD model to the PD data are provided in Figure S3.

The effect of capsaicin administration was estimated at a 2.69-unit increase in DBF, suggesting a nearly 3-fold increase in DBF from the pre-capsaicin baseline, while the change in DBF associated with vehicle alone was estimated at only -0.0431, indicating only a very slight decrease (approximately 4%) in the DBF response from time 0 to 30 minutes post-administration of vehicle. The effect of placebo on the change in capsaicin-induced DBF was a small reduction of 0.193 (approximately 7%). The I_{max}, expressed as a fractional decrease in the capsaicin-induced DBF due to galcanezumab, was 0.705 and the IC_{50} was estimated at 1060 ng/mL. From the typical baseline change in capsaicin-induced DBF of 2.69, the maximum drug effect represents a reduction of 1.9 units (or 71%). The estimate of delay parameter k_{e0} was 0.375 $days^{-1}$, representative of a half-life of equilibration between the serum and effect compartments of 1.8 days. Although the IC_{50} estimate (1060 ng/mL) represents the concentration in the effect compartment associated with 50% of the maximal response (not the central compartment concentration), this value is in the range of the peak serum concentrations achieved with a 25-mg single dose and well below serum concentrations achieved following 75 mg or more for approximately 75 days after the dose. Simulations of predicted effect compartment concentrations were very similar in magnitude to the corresponding central compartment concentrations.

The magnitude of the unexplained BSV in PK/PD parameters was relatively large for some parameters and smaller for others, with 329 %CV (coefficient of variation expressed as a percentage) for k_{e0}, 155 %CV for IC_{50}, 89 %CV for k_a, approximately 69 %CV for vehicle effect, 29 %CV for baseline capsaicin-induced DBF, 27 %CV for CL/F, 21 %CV for V/F, and only 8 %CV for I_{max}. The RV was estimated with standard deviations (STD) of 0.299 log-galcanezumab concentration units, 0.09 for vehicle DBF, and 0.49 for capsaicin-induced DBF measurements.

Figure S4 illustrates the pcVPC results for the PK/PD model, with the median and 90% prediction intervals from the simulated datasets (capsaicin and vehicle administration shown separately) overlaid on the observed change from baseline in DBF versus time data. The majority of the observed data falls within the prediction interval, with an appropriate amount and similar distribution of observed data points falling above and below. Furthermore, the central tendency of the data, in general, appears to be adequately described by the model, as the median line for the simulated data bisects the observed data over the entire time interval, thus supporting the appropriateness of the model.

Simulation Results

Using the final PK/PD model, simulations of 1000 virtual subjects were performed for placebo and 6 galcanezumab dosing rates (0.5, 1, 2.5, 5, 15, and 75 mg/week for 1 year) with 2 different dosing intervals/treatment durations (q2wk × 7 doses and 13 weeks apart [q13wk] × 2 doses) including random BSV and RV.

Figure S5 provides the median predicted effect compartment concentration versus time profiles for each simulated dose regimen, with a line superimposed at the predicted estimate of the IC_{50} (1060 ng/mL). For the q2wk regimens, the 15- and 75-mg/wk dosing rates maintain a median

concentration above the IC_{50} for most of the time during the 7-week dosing period, while the 5-mg/wk dosing rate achieves a median concentration above the IC_{50} after 3 doses that falls below the IC_{50} by approximately 20 weeks. For the q13wk regimens, the 5-, 15-, and 75-mg/wk dosing rates all maintain a median concentration above the IC_{50} for most of the time during the 26-week dosing period, while the 2.5-mg/wk regimen achieves a median concentration above the IC_{50} for approximately 5 weeks during each 13-week dosing interval. Only a very slight degree of accumulation in the predicted effect compartment concentration is observed with the second dose administration of the q13wk regimen, while a much greater degree is evident over the time period of the 7 doses administered q2wk.

Figure S6 provides the median and 80% prediction interval about the predicted change from baseline in capsaicin-induced DBF at specified times following dose administration versus dosing rate for each simulated dose regimen. These plots illustrate (i) the similarity in the predicted response following the 2 highest dosing rates evaluated (15 and 75 mg/wk) regardless of regimen, (ii) a slightly greater response with similar dosing rates at 12 to 13 weeks following the start of the q2wk regimen as compared to the q13wk regimen, and (iii) a greater response at 24 to 26 weeks after the initiation of the q13wk regimen as compared to the q2wk regimen (although this can be attributed to the length of time off therapy at 24 weeks for the q2wk regimen).

Figure 6 shows the effect of dose on the change from placebo in reduction of migraine headache days in episodic migraine patients superimposed on the relationship between dose and model-predicted change from baseline on inhibition of capsaicin-induced increases in DBF. Despite the differences in scale and magnitude of the migraine and DBF responses, the overall relationships between dose and these PD responses are similar. Both 120 and 300 mg of galcanezumab

monthly for 3 months produced similar statistically significant (P = 0.02) reductions in the mean numbers of headache days compared to placebo and were predicted to have maximum inhibitory effects on capsaicin-induced increases in DBF, corresponding to the plateau portion of the exposure-DBF curve.[19] The lower doses of 5 and 50 mg did not achieve significant reductions in migraine headache days and were, similarly, not predicted to have maximum inhibitory effects on capsaicin-induced increases in DBF as these doses correspond to the steeper (non-plateau) portion of the dose-response curve.[35]

Discussion

The approval of monoclonal antibodies that bind CGRP or the CGRP receptor, as well as of small-molecule CGRP receptor antagonists (ie, the gepants), for the treatment of migraine provides strong evidence that inhibition of CGRP-driven pathophysiological processes resulting from activation of the trigeminovascular system provides a novel therapeutic approach in migraine treatment.[8,9,13-16,36] Galcanezumab is a humanized monoclonal antibody that binds to CGRP ligand and has been approved for migraine prophylaxis as of September 2018.[16] This manuscript describes a modeling and simulation approach that was applied in early clinical development to characterize the relationship between galcanezumab serum concentrations and the capsaicin-induced DBF response. Development of this PK/PD model supported a simulation-based evaluation of the capsaicin-induced DBF response in healthy subjects after various dosing regimens. Previous studies have provided a sound physiological basis for the use of CIDBF as a biomarker to assess engagement of the CGRP receptor.[18,24,26,37,38]

After administration of single galcanezumab doses (1 to 600 mg), maximum serum concentrations were generally achieved by 5 to 14 days, consistent with slow absorption from the subcutaneous tissue. The elimination phase of the concentration-time profile after single or

multiple doses generally appeared mono-exponential and dose proportional across the range of single doses. Galcanezumab concentrations tended to be higher after multiple-dose administration relative to the single-dose cohorts, consistent with expected drug accumulation, based on the estimated elimination half-life of about 30 days in this study.

The PK of galcanezumab was described with a 1-compartment model with first-order absorption following a short lag time and first-order elimination. Over the range of doses in the study, the typical galcanezumab CL/F was estimated to be 0.0106 L/h and the typical V/F was 11.2 L, which are consistent with what was reported previously for patients with episodic migraine, as well as PK parameters for other IgG monoclonal antibodies.[39,40]

An effect compartment (biophase) link model was developed to describe the relationship between galcanezumab serum concentrations and change from baseline in DBF response following capsaicin or vehicle administration. A biophase model accounts for the delay between the attainment of peak serum concentration and the maximum response (in this case, the maximum reduction in the change from baseline in capsaicin-induced DBF response) and describes the inhibitory effect of galcanezumab via a saturable (I_{max}) function. This model has been used previously to relate PK and DBF responses for galcanezumab in non-human primates to predict human doses, and was found to adequately characterize the time-course and exposure-response relationship evident in the data from this trial.[18] Although an indirect response model could have been considered since these data exhibit an apparent delay between the peak PK and PD responses, exploratory graphs did not support the indirect response model assumption that the time of the maximum response increases with increasing dose.[41] Similar PK/PD link modeling approaches have also been used for the monoclonal antibody targeting the CGRP receptor, erenumab, and the CGRP antagonist monoclonal antibody, eptinezumab.[42]

Previous reports have described results for inhibition of capsaicin-induced DBF by the oral CGRP receptor antagonists telcagepant and MK-3207.[24,26] However, a double I_{max} model was required to additionally account for the 2 dose levels of capsaicin (300 μg and 1000 μg) in the telcagepant and MK-3207 trials. The fractional I_{max} of approximately 92% achieved for both telcagepant and MK-3207, and the fractional maximum DBF inhibition reported for AMG334 of approximately 90%, are slightly larger than that reported in the current analysis (71%), however, the difference is too small to make a definitive inference.[24,26,27,43,44] Collectively, it is safe to conclude that the action of CGRP on the CGRP receptor is the predominant contributor to capsaicin-induced DBF response.

The effect compartment concentration associated with 50% of the maximal reduction in capsaicin-induced DBF (IC_{50}) was estimated at 1060 ng/mL (or approximately 7.3×10^{-9} mol/L), a value 2 to 3 orders of magnitude greater than the in vitro potency of 30×10^{-12} mol/L. Given the differences in conditions between in vitro experiments and the in vivo milieu, as well as the influence on in vivo potency of the kinetics of target turnover and drug concentration, such in vitro to in vivo potency differences are not considered uncommon. In relation to galcanezumab exposure and dose, the estimated IC_{50} is on the lower end of the range of peak concentrations (measured in the central compartment) associated with a 25-mg single dose and well below central compartment concentrations achieved with a 75-mg single dose for up to 75 days after dosing. Central compartment concentrations following the multiple-dose 150-mg q2wk regimen (total of 4 dose administrations) tended to be well above the IC_{50} estimate for the entire PK sampling period (176 days). Thus, the estimate of IC_{50} for galcanezumab, although associated with a large degree of BSV, is well within the range of central compartment concentrations

achieved with the doses evaluated in this trial (and simulated effect compartment concentrations expected with these regimens), providing confidence in the estimate.

The precision of PK/PD model parameter estimates was only moderate and BSV was high, especially for k_{e0} and IC_{50}. These findings are not unexpected based on the relatively small sample size and informational content in the data relative to some estimated parameters. This high degree of BSV in these parameters did contribute to the relatively wide prediction intervals about the expected responses derived from the simulations.

Two galcanezumab dosing regimens with 6 galcanezumab dosing rates and placebo treatment were simulated for a large virtual population. Simulation results showed larger fluctuation in the response profile (from placebo to higher exposures) following the less frequent q13wk regimen, as compared to the q2wk regimen (Figure S6); however, at a dosing rate of 15 mg/week (195 mg q13wk) or higher, the fluctuation in the PD response is relatively small, indicating the possibility that inhibition of capsaicin-induced DBF may be maintained with less frequent dosing. The results of this modeling effort demonstrated that the doses that achieve a maximum effect on inhibition of DBF are also the doses that demonstrate some clinical efficacy. Consequently, doses that do not produce a maximal change in DBF may not be sufficiently large to show efficacy in inhibiting migraine attacks.

Simulation results showed little increase in response for dosing rates higher than 15 mg/wk (Figure S6), regardless of dose frequency, suggesting a saturation of the capsaicin-induced DBF inhibitory effect of galcanezumab. Doses of at least 15 mg/wk q2wk or 5 mg/wk q13wk are needed to maintain concentrations above the IC_{50} over most of the dosing interval as shown in Figure S5. However, the highest dosing rate of 75 mg/wk, administered q13wk was associated with a median predicted peak serum concentration of approximately 80,000 ng/mL, which is

considerably higher than the maximum observed concentration attained following the doses administered in this study (approximately 60,000 ng/mL).

Importantly, it should be noted that galcanezumab did not alter basal DBF. This observation is consistent with several findings showing that although CGRP is a potent vasodilator, it is not a factor in maintaining vascular tone in the resting, or basal, condition.[28,30] Other studies employing galcanezumab, erenumab, or the small-molecule CGRP antagonists olcegepant and telcagepant likewise showed no effect on resting blood flow.[28,45-47] Consistent with this, galcanezumab did not produce clinically meaningful changes in blood pressure or pulse rate in phase 3 clinical trials.[48]

Based on the use of changes from baseline in vehicle- and capsaicin-induced DBF as measured by LDI as the biomarker representative of PD response, the PK/PD model described herein provides evidence for exposure-dependent CGRP binding and neutralization. As shown in Figures S5 and S6, simulations of 150 mg galcanezumab q2wk (75 mg/wk) for 14 weeks are predicted to result in median concentrations well above the estimated IC_{50} for over 30 weeks and a near maximal response in change from baseline CIDBF. In a phase 2 proof-of-concept study conducted in a population of migraineurs with a relatively high frequency of headache days per month, 150 mg galcanezumab (or placebo) q2wk for 12 weeks was evaluated. Under this regimen that would be expected to result in very high levels of inhibition of CIDBF based on the simulations, galcanezumab showed superior efficacy over placebo in the number of migraine headache days despite a high placebo response.[1] This was borne out in phase 3 clinical trials with patients with episodic or chronic migraine who received a starting dose of 240 mg of galcanezumab and monthly doses of either 120 mg or 240 mg. [20-22] In the present investigation, we showed substantial overlap between galcanezumab doses predicted to have maximal

inhibition of capsaicin-induced increases in DBF and doses that significantly reduce headache days (Figure 6).[19] This observation suggests that inhibition of capsaicin-induced elevation in DBF may be a useful biomarker to aid in dose selection for migraine efficacy trials where CGRP is a putative mechanism related to the disease indication. An understanding of the correlation between capsaicin-induced DBF and migraine efficacy will continue to develop as more clinical efficacy data are generated and our understanding of the role of CGRP in migraine expands.

Conclusion

Overall, PK/PD modeling and simulation provided important quantitative understanding of dose- and exposure-response relationships in early clinical development to facilitate development planning decisions, including the selection of dose for the multiple-dose phase of the study and support for regimen selection decisions in future trials.

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Figure Legend

Figure 1. Schematic of the effect compartment pharmacokinetic/pharmacodynamic link model. tlag, absorption lag time; Caps, capsaicin; Ce, effect compartment concentration; CL/F, apparent clearance; Cp, serum concentration; k_{1e}, first-order transfer rate constant for drug into the effect compartment; k_a, absorption rate constant; k_{e0}, first-order transfer rate constant for drug out of the effect compartment (and between the plasma and effect compartments at equilibrium); SC, subcutaneous; V_c, volume of the central compartment; Veh, vehicle.

Figure 2. Prediction intervals of simulated data overlaid on the observed galcanezumab concentrations for the final pharmacokinetic model.

Figure 3. A single dose of galcanezumab (200 mg) inhibits capsaicin-induced increased dermal blood flow (DBF). Laser Doppler imaging shows that capsaicin induces marked increases in DBF in the placebo-treated subjects 30 minutes after application. The capsaicin-induced increase in DBF is markedly attenuated in subjects receiving galcanezumab.

Figure 4. Mean profiles of the change from baseline in capsaicin-induced increase in dermal blood flow (DBF) versus day for each dose group. MD, multiple doses; SD, single dose.

Figure 5. Scatterplot of change from baseline in capsaicin-induced increase in dermal blood flow (DBF) versus galcanezumab concentrations, by dose, with regression line overlaid to illustrate the central tendency of the measurements across the range of exposure values. MD, multiple doses; SD, single dose.

Figure 6. The reduction in mean headache days is shown juxtaposed with the predicted change in baseline DBF across a 3-month dosing interval with 5, 50, 120, and 300 mg of galcanezumab. The black symbols represent the geometric least squares mean and standard error for change from placebo in migraine days. The red lines represent the model-predicted median (solid) and 90^{th} prediction interval (dashed) for DBF across a 3-month dosing interval.

Table 1. Parameter Estimates and Standard Errors for the Pharmacokinetic/Pharmacodynamic Model for Galcanezumab

	Final Parameter Estimate			Interindividual and Residual Variability				
Parameter	**Population Mean**	**%RSE**	**Bootstrap Median (95% CI)**	**Final Estimate**	**%RSE**	**Magnitude**	**Bootstrap Median (95% CI)**	**Model Form**
k_a: absorption rate constant (1/h)	0.0192	11.1	0.0190 (0.0155, 0.0244)	0.587	19.7	89.4 %CV	87.8%CV (65.9%, 114.8%)	Exponential
CL/F: apparent clearance (L/h)	0.0106	4.17	0.0106 (0.00980, 0.0115)	0.0679	35.0	26.5 %CV	25.7%CV (15.2%, 34.6%)	Exponential
V/F: apparent volume of distribution (L)	11.2	4.22	11.2 (10.4, 12.1)	0.0432	68.0	21.0 %CV	20.6 %CV (8.12%, 33.8%)	Exponential
$t_{1/2}$:half-life (days)	30.5	-	-	NE	NA	NA	-	NA
ALAG1: lag time for absorption (h)	0.202	24.5	0.204 (0.0972, 0.284)	NE	NA	NA	-	NA
log RV in PK	-	-	-	0.0893	17.2	0.299 STD	0.297 SD (0.250, 0.355)	Additive
k_{e0}: first-order transfer rate constant for drug between the plasma and effect compartments (1/day)	0.375	67.4	0.362 (0.0882, 2.846)	2.47	64.2	329 %CV	324 %CV (0%, 10510%)	Exponential
E0V: screening change in DBF for vehicle	-0.0431	14.4	-0.0435 (-0.0564, -0.0317)	8.85E-04	55.3	0.0298 STD	0.0287 STD (0.00112, 0.0427)	Additive
EVEH: shift in change in DBF for vehicle after day 0	0	FIXED	-	NE	NA	NA	-	NA
E0C: screening change in CIDBF	2.69	4.12	2.69 (2.46, 2.90)	0.607	19.4	0.779 STD	0.763 STD (0.594, 0.912)	Additive
I_{max}: maximum fractional decrease in change in CIDBF due to galcanezumab	0.705	6.91	0.720 (0.610, 0.812)	0.00341	357	0.0584 STD	0.0505 STD (2.55E-12, 0.124)	Additive
IC_{50}: galcanezumab concentration associated with 50% of maximum reduction (µg/mL)	1.06	43.1	0.878 (0.235, 2.357)	1.22	43.4	155 %CV	156 %CV (0.00438%, 1438%)	Exponential
EPLC: placebo effect on the CIDBF	-0.193	52.7	-0.145 (-0.362, 0.0309)	NE	NA	NA	-	NA
STD for additive RV on vehicle DBF (PK/PD model)	-	-	-	0.00858	11.8	0.0926 STD	0.0927 STD (0.0829, 0.104)	Additive
STD for additive RV on CIDBF (PK/PD model)	-	-	-	0.235	16.1	0.485 STD	0.476 STD (0.396, 0.556)	Additive

CI, confidence interval; CIDBF, capsaicin-induced dermal blood flow; %CV, coefficient of variation expressed as a percentage; DBF, dermal blood flow; IIV, interindividual variability; NA, not applicable; NE, not estimated; PK/PD, pharmacokinetic/pharmacodynamic; %RSE, percent relative standard error; RV, residual variability; STD, standard deviation.