

Several lines of evidence suggest a role for CGRP in migraine pathogenesis. First, elevated levels of CGRP have been detected in individuals with migraine (Goadsby et al., 1988, 1990; Gallai et al., 1995; Bellamy et al., 2006). Second, intravenous CGRP infusion produces lasting headaches in healthy individuals and migraine headache in migraineurs (Lassen et al., 2002; Petersen et al., 2004). Most importantly, several small-molecule CGRP-receptor antagonists have shown clinical efficacy in acute migraine and in migraine prevention. These include the peptide-like antagonist of the CGRP receptor olcegepant (BIBN4096BS), which showed a reversal effect on acute migraine pain that was indistinguishable from that of oral triptans (the standard of care) but without triptan-like adverse events (Olesen et al., 2004). Other small-molecule antagonists include BI 44370, which exhibited dose-dependent efficacy in the treatment of acute migraine (Diener et al., 2011); BMS-927711 (C₂₈H₂₈F₂N₆O₃), which showed efficacy in phase 2 trials (Marcus et al., 2014); and MK-0974 (telcagepant) and MK-3207, which showed efficacy in phase 2 trials (Ho et al., 2008b; Hewitt et al., 2011), two pivotal phase 3 trials of acute migraine (Ho et al., 2008a; Connor et al., 2009), and a study for migraine prevention (Ho et al., 2014). Together, these data provide sufficient evidence that blocking the CGRP signaling pathway is a sensible approach for the development of new migraine therapeutics. Unfortunately, despite the extensive efforts in pursuing small-molecule CGRP antagonists for the treatment of migraine, none of these agents has been approved for use as a result of poor pharmacokinetic properties (olcegepant) or liver toxicity concerns (MK-0974 and MK-3207) (Hewitt et al., 2011).

A monoclonal antibody may be a preferred modality to target the CGRP receptor, especially for the prophylactic treatment of migraine, for several reasons: 1) the ligand-binding site of CGRP on the calcitonin receptor-like receptor (CRLR)-receptor activity-modifying protein-1 (RAMP1) CGRP-receptor complex is broad (Hollenstein et al., 2014); therefore, an antibody that can span the distance between these receptor subunits can offer the most effective blockade; 2) high selectivity for the CGRP receptor complex, which shares similarities with other receptors in the family, and if these other receptors are blocked by a less selective agent, undesired side effects could arise; and 3) the prolonged serum half-life of an antibody can permit longer dosing intervals, which are ideal for preventive therapies. Using XenoMouse technology (Amgen, Inc., Thousand Oaks, CA), we successfully generated multiple human monoclonal antibodies that interact with the CGRP receptor. The aim of these studies was to characterize the *in vitro* and *in vivo* pharmacologic properties of the CGRP-receptor antagonist AMG 334, which is currently in clinical development for migraine prevention.

Materials and Methods

Generation of the AMG 334 Antibody. XenoMouse were immunized with purified soluble CGRP receptor protein as the antigen. Soluble CGRP receptor polypeptides containing the N-terminal extracellular domains (ECDs) of human CRLR (amino acids 1–138 of GenBank accession no. AAA62158) and human RAMP1 (amino acids 1 to 117 of GenBank accession no. CAA04472) were generated by transiently cotransfecting 293 6E cells. Hybridomas are generated from a pool of mice with the highest sera titer using a standard protocol (Kearney et al., 1979) and AMG 334 is identified through

screening assays including binding competition, functional blocking, and receptor selectivity against the human CGRP receptor.

Cell Lines and Cell Culture. Human neuroblastoma cells (SK-N-MC) endogenously expressing human CGRP receptors were isolated from a 14-year-old girl with neuroepithelioma [American Type Culture Collection (ATCC), Manassas, VA (cat. no. HTB-10)]. Cells were cultured in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS), 1X glutamine/penicillin/streptomycin, 1X MEM nonessential amino acids (MEM NEAA), and 1X MEM sodium pyruvate. Human embryonic kidney 293 (HEK-293) cell line, stably expressing cynomolgus monkey (*cyno*) CGRP receptors, was generated by cotransfection of *cyno* CRLR and RAMP1 cDNAs into HEK cells (Protein Science, Amgen Inc., Thousand Oaks, CA), and clones were selected inhouse. The cells were grown in Dulbecco's modified Eagle's medium (DMEM), 5% FBS, 1× glutamine/penicillin/streptomycin, 1× MEM NEAA, 1× MEM sodium pyruvate, 200 μg/ml Zeocin, 250 μg/ml Geneticin (G418), and 200 μg/ml hygromycin. Rat L6 myoblast cells endogenously expressing rat CGRP receptors [ATCC, Manassas, VA (cat. no. CRL-1458)] were cultured in DMEM containing 10% FBS and 1× glutamine/penicillin/streptomycin. Human adrenomedullin-1 receptor stable cell line was generated by cotransfection of human CRLR and RAMP2 cDNAs into HEK/EBNA cells (Protein Science), and clones were selected inhouse. The cells were cultured in DMEM, 5% FBS, 1× glutamine/penicillin/streptomycin, 1× MEM NEAA, 1× MEM sodium pyruvate, 200 μg/ml blasticidin (Zeocin), 250 μg/ml G418, and 200 μg/ml hygromycin. Human adrenomedullin-2 receptor stable Chinese hamster ovary cell lines [PerkinElmer Life and Analytical Sciences, Waltham, MA (cat. no. ES-430-C)] were cultured in Ham's F12, 10% FBS, 1× glutamine/penicillin/streptomycin, 10 μg/ml blasticidin, and 400 μg/ml G418. HEK cells stably expressing human calcitonin receptors [Invitrogen, Inc., San Diego, CA (cat. no. K1437)] were cultured in DMEM with 10% FBS, 1× glutamine/penicillin/streptomycin, 1× MEM NEAA, 10 mM HEPES, 400 μg/ml G418, 100 μg/ml hygromycin, and 5 μg/ml blasticidin. Human MCF-7 epithelial cells endogenously expressing human amylin receptors were isolated from a 69-year-old woman with adenocarcinoma [ATCC, Manassas, VA (cat. no. HTB-22)]. Cells were cultured in Eagle's MEM containing 10% FBS, 1× glutamine/penicillin/streptomycin, 1× MEM NEAA, and 1× MEM sodium pyruvate.

Membrane Preparation and Binding Studies of AMG 334 on Human, Rabbit, and Dog CGRP Receptors. SK-N-MC cell membrane was purchased from PerkinElmer Life and Analytical Sciences (cat. no. RBHGRPM400UA). Dog and rabbit brain membranes were prepared inhouse. Briefly, dog and rabbit whole-brain slices were collected and cut into millimeter-sized pieces in a 100-mm tissue culture dish. The tissues were transferred to a 55-ml Wheaton glass homogenizer containing hypotonic buffer [20 mM Tris-HCl (pH 7.5) and 5 mM MgSO₄], and the samples were placed on ice for 30 minutes at 500 rpm. The tissues were homogenized, transferred to 50 ml conical tubes, and centrifuged for 10 min at 500 rpm. The middle phase containing proteins was transferred to a 50-ml centrifuge tube and centrifuged at 19,920 rpm for 12 minutes in a Beckman Avanti J-26 XPI centrifuge (Beckman Coulter, Inc., Brea, CA). After removing the supernatant, the pellet was resuspended in hypotonic buffer and homogenized. The sample was centrifuged again at 19,920 rpm for 12 minutes, and the pellet was resuspended in buffer. Membrane aliquots were stored at –80°C for future use.

A radioligand binding assay was used to study the binding affinity of AMG 334 at human, rabbit, and dog CGRP receptors using [¹²⁵I]-CGRP radioligand [PerkinElmer Life and Analytical Sciences (cat. no. NEX3540)]. The binding assay was performed in a 96-well format. SK-N-MC cell membrane (10 μg/well) and rabbit and dog brain membranes (50 μg/well) were thawed and resuspended in assay buffer (20 mM Tris-HCl pH 7.5, 5 mM MgSO₄, 0.2% bovine serum albumin, and complete protease inhibitor). Cell homogenate was incubated with 50 pM [¹²⁵I]-CGRP and increasing concentrations of unlabeled AMG 334 (0.5 pM to 10 μM) for 3 hours at room temperature at 60 rpm shaking speed. The assay plates were filtered through 0.5%

polyethyleneimine-treated GF/C (glass fiber) filter plates using a cell harvester to terminate the reaction. The GF/C plates were washed six times with cold washing buffer (50 mM Tris-HCl). Next, the GF/C plates were dried in a 55°C oven for 1 hour; the bottoms of the plates were sealed, and 50 μ l of MicroScint-20 (PerkinElmer Inc., Waltham, MA) was added. After sealing the tops of the plates with press-on adhesive sealing film (TopSeal-A; PerkinElmer Inc.), they were counted with microplate scintillation and luminescence counter (TopCount NXT-Packard, model 0384V00; PerkinElmer Inc.), and data were analyzed using GraphPad Prism software version 5.1 (GraphPad Software Inc., La Jolla, CA).

Functional Studies of AMG 334 on Human, Cyno, and Rat CGRP receptors. SK-N-MC cells endogenously expressing CGRP receptors (Muff et al., 1992) and a functional cAMP accumulation assay were used to measure the agonist and antagonist activity of AMG 334. Agonist activity of AMG 334 was tested by incubating with SK-N-MC cells for 30 minutes at room temperature (5 pM to 10 μ M); 0.5 pM to 1 μ M of the α -form of CGRP, the most abundant form of CGRP in the body (hereafter referred to as CGRP), was used as a positive control. The reaction was stopped by adding detection mix to all wells, followed by additional 60-minute incubation at room temperature. The assay plates were read on an EnVision instrument (Software version 1.07, PerkinElmer, Inc.) at an emission wavelength of 665 nm and data were analyzed using GraphPad Prism software version 5.1 (GraphPad Software Inc.). A similar procedure was used in the antagonist assays by using a fixed concentration of CGRP. The concentrations of CGRP were chosen based on its EC₅₀ values at CGRP receptors in SK-N-MC cells. A suspension of SK-N-MC cells was mixed with Alexa-labeled anti-cAMP antibody (1:100 final dilution of stock solution from LANCE assay kit) and added into 96-well half-area white plates (10,000 cells/well). AMG 334 (0.5 pM to 1 μ M) or CGRP₈₋₃₇ (0.5 pM to 1 μ M), a CGRP-receptor antagonist used as a positive control, was added and incubated for 30 minutes at room temperature. CGRP (1 nM final concentration) was then added and further incubated for 15 minutes at room temperature. The reaction was stopped, and the plates were read as described previously herein. The same method was used to measure the ability of AMG 334 to inhibit CGRP-induced cAMP accumulation in HEK cells stably expressing cyno CGRP receptors and L6 cells endogenously expressing rat CGRP receptors. CGRP peptide was used as the agonist in both assays.

Activity of AMG 334 on Human Adrenomedullin, Calcitonin, and Amylin Receptors. Antagonist activity of AMG 334 was studied in HEK cells stably expressing human adrenomedullin-1 receptors, Chinese hamster ovary cells stably expressing the human adrenomedullin-2 receptor, HEK cells stably expressing the human calcitonin receptor, and MCF-7 cells endogenously expressing human amylin receptors. The same cAMP functional assay described already in this article was used to determine the activity of AMG 334. Adrenomedullin peptide was used as the agonist in adrenomedullin-1 and adrenomedullin-2 receptor assays, and calcitonin peptide was used as the agonist in calcitonin and amylin receptor assays.

Pharmacodynamic Activity of AMG 334. Adult male cynos were cared for, and all procedures in this study complied with the Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health, and the Office of Laboratory Animal Welfare. All procedures were approved by the institution's Animal Care and Use Committee. The cynos were provided by Valley Biosystems (West Sacramento, CA) and housed individually in their facility in unidirectional airflow rooms with controlled temperature (18–26°C) and relative humidity (30%–70%), and 12-hour light/dark cycles (0600–1800). Potable water was available ad libitum. Cynos were fed a standard nonhuman primate (NHP) diet (Harlan Teklad 2050; Indianapolis, IN) twice daily (AM and PM). All animals had a prestudy physical examination, including complete blood count and serum chemistry. Only animals with normal blood parameter values were used in this study. All animals were fasted the night before the laser Doppler measurements.

Prestudy Preparation. Animals were anesthetized initially with ketamine (10 mg/kg intramuscular), placed in a supine position on a temperature-controlled water circulating blanket, and intubated with a 4-mm tracheal tube connected to an oxygen/isoflurane gas anesthesia system (3%–4% isoflurane for induction, 1.5%–2% for maintenance). Isoflurane levels were adjusted to maintain consistent heart rate and blood oxygen levels. To maintain a stable body temperature, an additional hot-air blanket was used to cover each animal's body. Before the laser Doppler study, a catheter was temporarily placed in the saphenous vein for i.v. drug infusion. Before each testing session, the animal's skin was shaved on the ventral surface of either the forearm or medial thigh.

Capsaicin-Induced Dermal Blood Flow: Dose-Response Study Design. A laser Doppler imager (model no. LDI-2, Moor Instruments, Ltd, Wilmington, DE) was used to measure dermal blood flow (DBF) on the skin of the ventral forearm or the medial thigh of cynos. This measurement of increase in DBF with topical application of capsaicin in cynos was carried out as previously reported in rhesus monkeys (Hershey et al., 2005; Salvatore et al., 2008) with a minor change (i.e., O rings were specifically placed on areas of low baseline blood flow to maximize the dynamic window of the capsaicin response). After demonstrating that responses after 1 mg or 2 mg were not statistically distinguishable (data not shown), we proceeded with 1 mg of capsaicin. On day 0, baseline DBF was assessed on one of four potential predetermined limbs (arm or leg) of anesthetized cynos followed by topical application of capsaicin at 1 mg in 20 μ l and laser Doppler scans every 5 minutes for 30 minutes after capsaicin application. Cynos then received one of five different doses of AMG 334 (0.1, 0.3, 3, 10, and 30 mg/kg; $n = 6$) or 4 mg/kg of the control antibody anti-dinitrophenol (α DNP; a human IgG2 nonspecific control domain antibody "dummy"; $n = 8$) using a modified counterbalanced design. After dosing, animals were allowed to recover and returned to their home cages. On day 2, animals were again anesthetized and prepared for a postdrug baseline DBF measurement on a limb different from the one used on day 0, followed by capsaicin topical application and laser Doppler scans every 5 minutes for 30 minutes after capsaicin application. After completion of the testing session, animals were allowed to recover and returned to their home cages. On day 4, the same testing procedure was repeated as on day 2, with each animal tested only three times: before drug-capsaicin challenge (day 0), 2 days after drug-capsaicin challenge (day 2), and 4 days after drug-capsaicin challenge (day 4). Six of the aforementioned cynos dosed with α DNP were also tested on day 0, day 2, and day 7 (but not on day 4). One group of cynos was dosed with 4 mg/kg of AMG 334 ($n = 6$) and evaluated for capsaicin response on day 0, day 2, and day 7.

Blood Sample Collection and Pharmacokinetic Analysis. Whole-blood samples for pharmacokinetic analysis (1 ml) were collected from all animals at multiple time points, including day 0, immediately after the 30-minute infusion and immediately after DBF testing on days 2 and 4. The blood was collected via direct puncture of the cephalic, femoral, or saphenous vein. AMG 334 and α DNP concentrations in serum were measured using an enzyme-linked immunosorbent assay. The lower limit of quantification for the method was 1 ng/ml for AMG 334 and 20 ng/ml for α DNP.

Statistical Analyses. All statistical analyses were completed using SAS V9.1.3. We examined the ratio of the post-capsaicin challenge flux measurement to its corresponding pre-capsaicin challenge value (referred to as the *original ratio*) and the geometric mean of the 20, 25, and 30 post-capsaicin challenge flux measurement normalized (divided by) to the pre-capsaicin challenge value (referred to as the *composite ratio*). Analysis and corresponding graphical representation of this data using composite ratio as opposed to percent change from baseline serve as a form of normalization that increases statistical power. To examine the dose-response relationship of AMG 334, the median response was found and reported for each day by dose combination. A *P*-value was then determined using the Jonckheere-Terpstra trend test, which tests for a monotone trend, for each day of measurement. The endpoints used for the trend test were preadjusted

by the 0-minute pre-capsaicin measure as well as the day 0 measure. The adjustment was done by using the slope of covariates from a mixed-effect model. A similar analysis was performed for the original ratio at each time point. A mixed-effect analysis of covariance on the composite endpoint was also performed to examine the dose-response relationship. Covariates included the natural log of the pre-capsaicin measure as well as the natural log of the measure on predose. Lastly, a nonlinear mixed-effect model of an E_{max} type with a hill coefficient and dose as the independent variable was performed. Parameters were estimated separately for day 2 and day 4. Covariates included the natural log of the pre-capsaicin measure as well as the natural log of the measure on predose. Mathematically, this model can be expressed in eq. 1:

$$(comp\ ratio)_{ij} = \left[a + \frac{Emax_j \cdot (dose_i)^{b_j}}{(ED50_j)^{b_j} + (dose_i)^{b_j}} \right] \exp(c \cdot \ln precap_{ij} + d \cdot \ln predose_i + \phi_i + \epsilon_{ij}) \quad (1)$$

where a is the intercept, b is the Hill 'coefficient', c and d are slope parameters, and ϕ and ϵ are the intersubject and intrasubject errors, respectively; $\ln precap$ is the natural logarithm (ln) of the pre-capsaicin measurement, and $\ln predose$ is the ln of the predose measurement; i indexes the animal, j indexes the day, and ϕ_i and ϵ_{ij} are both independent and normally distributed with mean 0 and variances ϕ^2 and σ^2 , respectively. Because the studies were not powered a priori, emphasis should be placed on the one-sided P -values when judging the strength of evidence for a treatment effect.

Results

AMG 334 Antagonist and Agonist Activity. AMG 334 potently and competitively inhibited the binding of [125 I]-CGRP to human CGRP receptors (SK-N-MC cells), with a K_i (S.D.) of 0.02 ± 0.01 nM ($n = 4$; Fig. 1). The peptide CGRP-receptor antagonist CGRP₈₋₃₇, a positive control used in the assay, displayed a K_i value of 2.9 ± 1.6 nM ($n = 4$). AMG 334 also showed a potent and full antagonistic effect in a functional assay, with an IC_{50} of 2.3 ± 0.9 nM ($n = 8$) in inhibiting CGRP-induced cAMP accumulation in SK-N-MC cells endogenously expressing human CGRP receptors and a maximal inhibition of $91.7\% \pm 1.8\%$ ($n = 8$). CGRP₈₋₃₇ displayed an IC_{50} value of 3.9 ± 2.0 nM ($n = 8$), with a maximum inhibition of $93.3\% \pm 2.0\%$. AMG 334 did not show

any agonist activity, even at the highest concentration tested ($10 \mu\text{M}$), whereas CGRP displayed an EC_{50} of 0.67 ± 0.58 nM ($n = 4$; Fig. 2, A and B).

Species and Receptor Family Selectivity. The activity of AMG 334 at CGRP receptors in cynos, rats, rabbits, and dogs was determined using either cell-based functional cAMP assays or radioligand binding assays similar to the methods described here. Antagonist potency of AMG 334 at the cyno CGRP receptor was similar to that at the human receptor, with an IC_{50} (S.D.) of 5.7 ± 2.8 nM ($n = 4$). No antagonist activity was observed for AMG 334 at rat CGRP receptors at the highest tested concentration of $10 \mu\text{M}$ ($n = 4$; Fig. 3A). AMG 334 showed measurable but markedly reduced binding potency to the rabbit and dog CGRP receptors in [125 I]-CGRP binding assays, with K_i of 230 ± 130 nM ($n = 3$) and 260 ± 130 nM ($n = 6$), respectively (Fig. 3B).

AMG 334 had no antagonist activity at the human adrenomedullin, calcitonin, and amylin receptors up to the highest concentration ($10 \mu\text{M}$; Table 1). The agonist activity of AMG 334 at these receptors was also evaluated and no agonist activity was observed up to the highest concentration tested ($10 \mu\text{M}$; data not shown).

Dose-Response Inhibition of Capsaicin-Induced Increase in DBF by AMG 334. Topical application of capsaicin (1 mg) produced an increase in DBF from 5 to 30 minutes relative to the baseline (pre-capsaicin), with an 88% increase at 30 minutes after application (Fig. 4). AMG 334 dose-dependently prevented capsaicin-induced increases in DBF, with a monotonic dose-dependent effect on day 2 ($P = 0.01$ for the composite ratio; original ratio at each 5-minute laser Doppler measurement from 10 to 30 minutes post-capsaicin with one-sided trend test $P < 0.05$; Fig. 5A). The magnitude and statistical significance of percent inhibition for each dosing group were evaluated relative to the combined data from the α DNP dosing group on day 2, day 4, and day 7 since it was determined that there was no significant difference in the capsaicin response as evaluated using the least-square geometric means of composite ratio ($P = 0.94$). AMG 334 also dose-dependently prevented capsaicin-induced increase in DBF on day 4 ($P = 0.0014$ for the composite ratio; $P < 0.05$ for the original ratio at each 5-minute laser Doppler

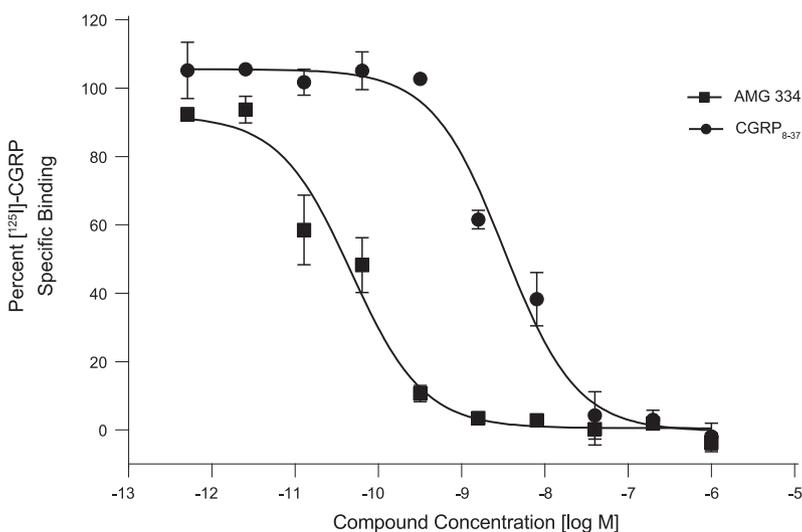


Fig. 1. Dose-dependent inhibition of [125 I]-CGRP binding to human neuroblastoma (SK-N-MC) cell membranes by AMG 334. CGRP₈₋₃₇ was used as a positive control. Figure is a representative curve from one of the experiments run in duplicate; error bars represent the S.D.

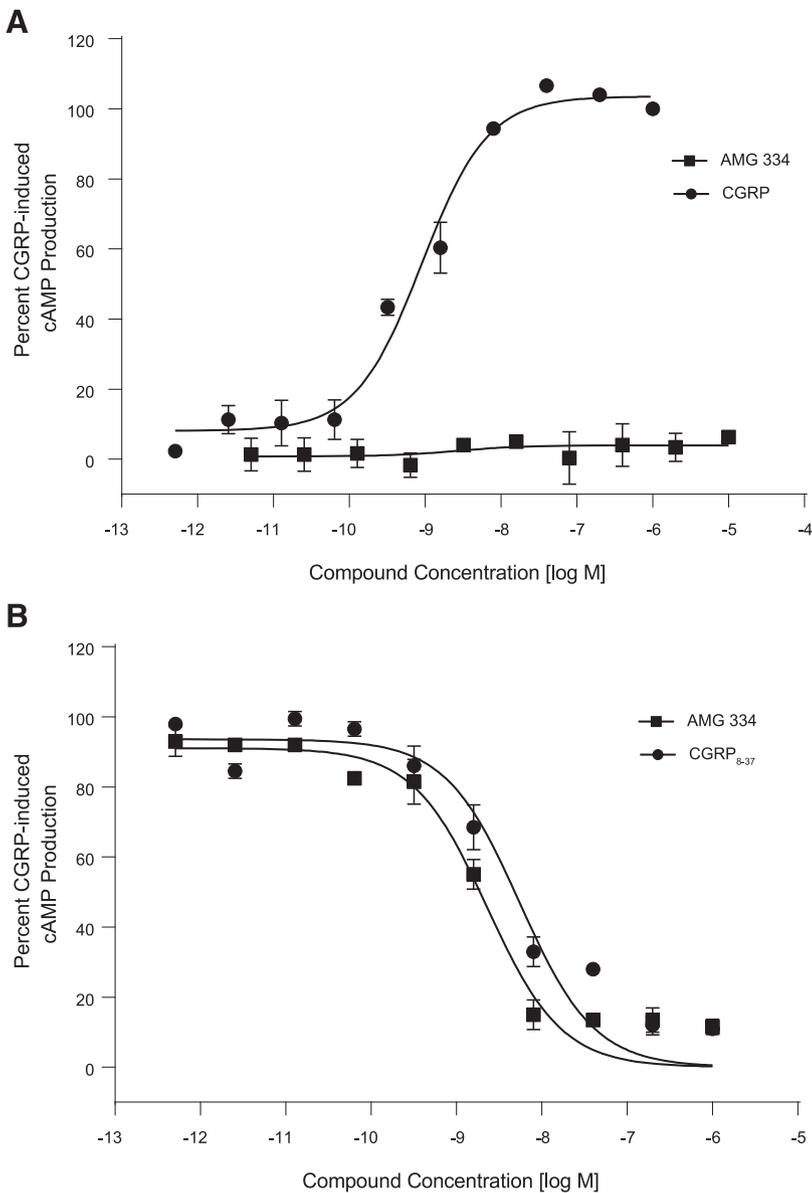


Fig. 2. AMG 334 potently antagonizes the CGRP receptor with no intrinsic agonist activity. The agonist and antagonist activities of AMG 334 were studied using human neuroblastoma (SK-N-MC) cells. (A) Agonist dose-response curve of AMG 334 in SK-N-MC cells with CGRP used as a positive control. (B) Antagonist dose-response curve of AMG 334 in SK-N-MC cells with CGRP₈₋₃₇ used as a positive control. Figures are representative curves from one of the experiments run in duplicate; error bars represent the S.D.

measurement from 5 to 10 minutes and 20 to 30 minutes with one-sided trend test; Fig. 5B). Based on the E_{max} model of predicted least-square geometric means of composite ratio, at 0.1 mg/kg, there was no significant effect in prevention of capsaicin-induced increase in DBF on day 2 (9.2% inhibition; one-sided $P = 0.34$) or day 4 (5.7% inhibition; one-sided $P = 0.39$). The lowest dose that produced a statistically significant effect was 0.3 mg/kg on day 2 (37.3% inhibition; one-sided $P = 0.05$) but not on day 4 (23.1% inhibition; one-sided $P = 0.17$). At 3 mg/kg, AMG 334 produced a statistically significant and also maximal response on day 2 (40.9% inhibition; one-sided $P = 0.0098$) and on day 4 (38.7% inhibition; one-sided $P = 0.012$). Doses greater than 3 mg/kg (4, 10, and 30 mg/kg) also produced significant and maximal (one-sided $P = 0.0098$ –0.015; 39–41%) responses on day 2 and day 4. The calculated estimate of the ED_{50} (95% confidence interval) was 0.15 mg/kg (0.03, 0.74) for day 2 and 0.25 mg/kg (0.03, 2.13) for Day 4. The resulting mean serum concentrations from samples taken immediately following DBF measurements on day 2 and day 4 for

each dose of AMG 334 are plotted on the right hand side y -axis of the DBF dose-response figures (Fig. 5, A and B). The mean C_{max} taken immediately after the 30-minute infusion of AMG 334 was approximately dose proportional over the dose range examined (0.1–30 mg/kg; data not shown).

Discussion

AMG 334 is the first human monoclonal antibody antagonist against the CGRP receptor. Like the small-molecule CGRP-receptor antagonists, AMG 334 fully antagonizes CGRP-receptor function and is dose-dependently effective in an in vivo target coverage model that exhibits translatability to humans. Whereas AMG 334 has a cell-based IC_{50} that is comparable to Merck's telcagepant (MK-0974), 2.3 nM versus 2.2 nM, respectively, the potency of AMG 334 in competing with [¹²⁵I]-CGRP binding is >20-fold greater than that of MK-0974 (K_i of 0.02 nM vs. 0.77 nM, respectively). This confirms the high specificity and affinity of antibody binding through

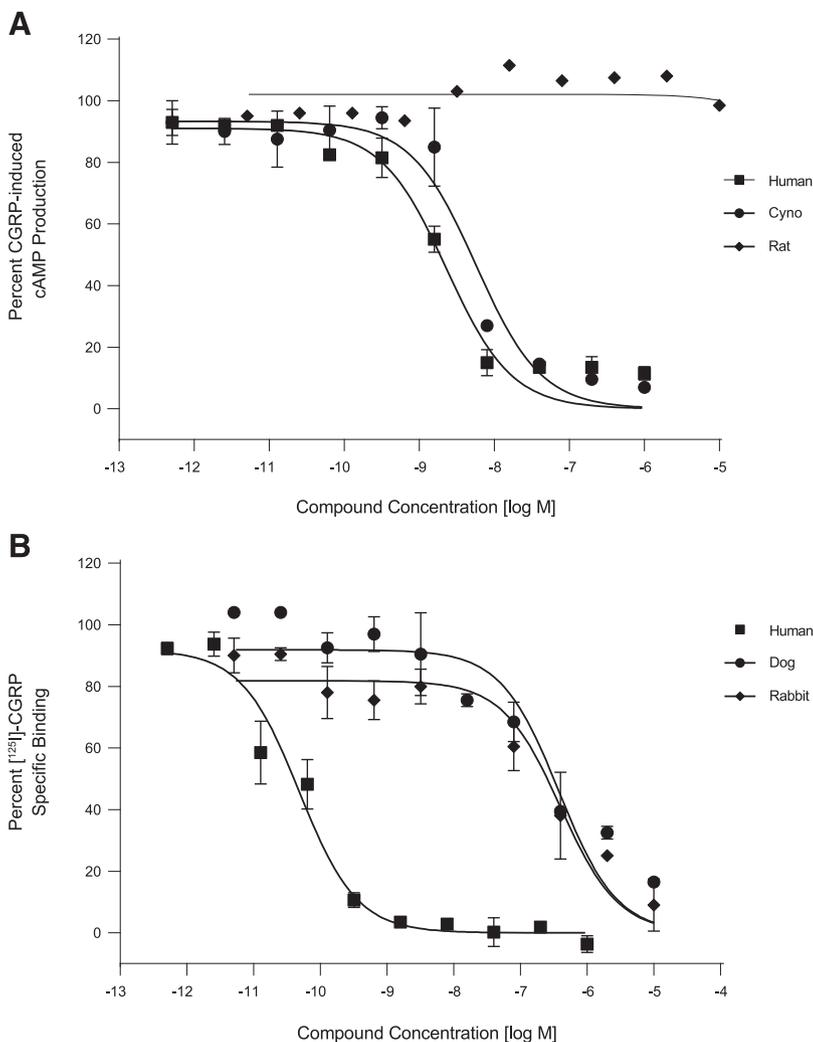


Fig. 3. AMG 334 selectively antagonizes cynomolgus monkey (cyno) CGRP receptors but not rat, rabbit, and dog CGRP receptors. (A) The functional potency of AMG 334 at cyno and rat CGRP receptors was studied in cell lines expressing cyno or rat CGRP receptors. Human neuroblastoma (SK-N-MC) cells were included as a positive control in the experiments. The antagonist activity of AMG 334 was determined by measuring inhibition of agonist-induced cAMP accumulation. (B) The dose-dependent binding potency of AMG 334 to rabbit or dog CGRP receptors was studied using rabbit or dog brain preparations. SK-N-MC membrane was included as a positive control in the experiments. Figures shown are representative curves from one of the experiments run in duplicate; error bars represent the S.D.

multiple surface binding interactions (Hollenstein et al., 2014). The specific nature of the ligand-receptor interaction between the radiolabeled AMG 334 antibody and the CGRP receptor, including competitiveness and reversibility, are discussed in a second manuscript, which is currently in preparation. The specificity is also supported by the receptor selectivity results as AMG 334 had no measurable activity (potency was >5000-fold higher than at the CGRP receptor) at all other receptors in the family up to the highest concentration tested in the assay (10 μ M); telcagepant, on the other hand, is highly selective for the CGRP receptor over the adrenomedullin receptors (CLR/RAMP2 and CLR/RAMP3),

with an $IC_{50} > 10 \mu$ M, but it is only modestly selective over the AMY1 receptor (CT/RAMP1), with an IC_{50} of 44 nM (Walker et al., 2015) and a binding K_i of 190 nM (Moore and Salvatore, 2012). Thus, compared with telcagepant, AMG 334 offers greater specificity at the CGRP receptor.

The species selectivity profiles of AMG 334 and MK-0974 are similar in that they both display similar affinity at the primate (human, cyno, and rhesus monkey) receptors: cell-based IC_{50} values of AMG 334 at the human and cyno CGRP receptors are 2.3 and 5.7 nM, respectively; the K_i values of MK-0974 at the human and rhesus monkey CGRP receptors are 0.77 and 0.78 nM, respectively. It is well documented that

TABLE 1

Antagonist activity of AMG 334 at human adrenomedullin, calcitonin, and amylin receptors

Human CGRP₈₋₃₇ peptide was used as the positive control for experiments involving the adrenomedullin-1 and adrenomedullin-2 receptors. Human calcitonin₈₋₃₂ peptide was used as the positive control for experiments involving the calcitonin and amylin receptors. IC_{50} values are expressed as mean \pm S.D. n = number of replicate experiments performed.

Cell Line	Human Receptor	Test Article, Positive Control	IC_{50} (nM)
AM1-HEK	Adrenomedullin-1	AMG 334, CGRP ₈₋₃₇ peptide	>10,000 ($n = 4$) 660 ± 370 ($n = 4$)
AM2-CHO	Adrenomedullin-2	AMG 334, CGRP ₈₋₃₇ peptide	>10,000 ($n = 4$) 420 ± 230 ($n = 4$)
Calcitonin-HEK	Calcitonin	AMG 334, calcitonin ₈₋₃₂ peptide	>10,000 ($n = 2$) 590 ± 210 ($n = 2$)
MCF-7	Amylin	AMG 334, calcitonin ₈₋₃₂ peptide	>10,000 ($n = 2$) 210 ± 10 ($n = 2$)

CGRP, calcitonin gene-related peptide; CHO, Chinese hamster ovary; HEK, human embryonic kidney.

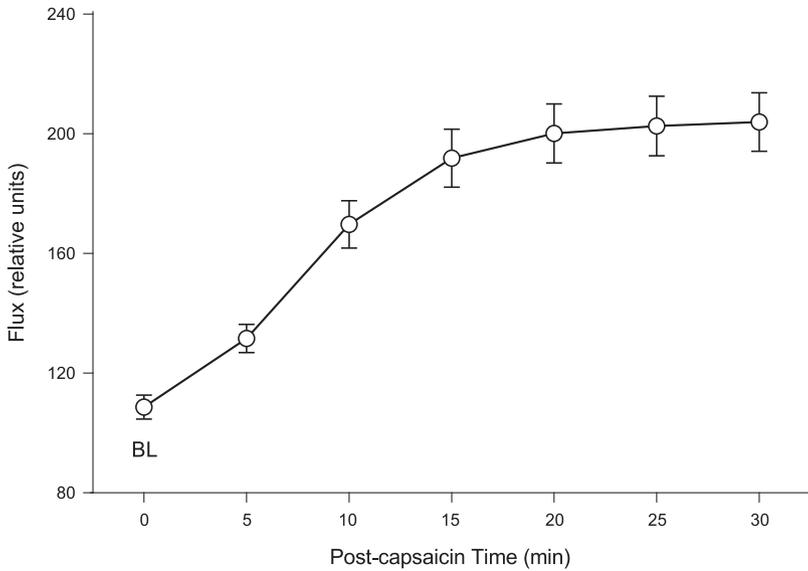


Fig. 4. Time course of capsaicin-induced increase in DBF as measured by laser Doppler imaging in cynos. DBF was measured by a laser Doppler imager. After obtaining baseline (BL) DBF, 1 mg of capsaicin solution was added topically into rubber O-rings, which were placed on the skin on the ventral side of the forearms or the medial thighs of cynos ($n = 44$). The increase in DBF (change from baseline) caused by topical application of capsaicin was expressed as flux (relative units). Data are expressed as the mean \pm S.E.M., and were obtained before dosing animals with i.v. infusions of AMG 334 or anti-dinitrophenol.

small-molecule antagonists of the CGRP receptor exhibit species-selective pharmacology (Doods et al., 2000; Edvinsson et al., 2001; Hasbak et al., 2001; Mallee et al., 2002), and here

we have shown that AMG 334, albeit a monoclonal antibody, is no exception. AMG 334 showed marked species selectivity, exhibiting >5000-fold higher affinity for the human and cyno

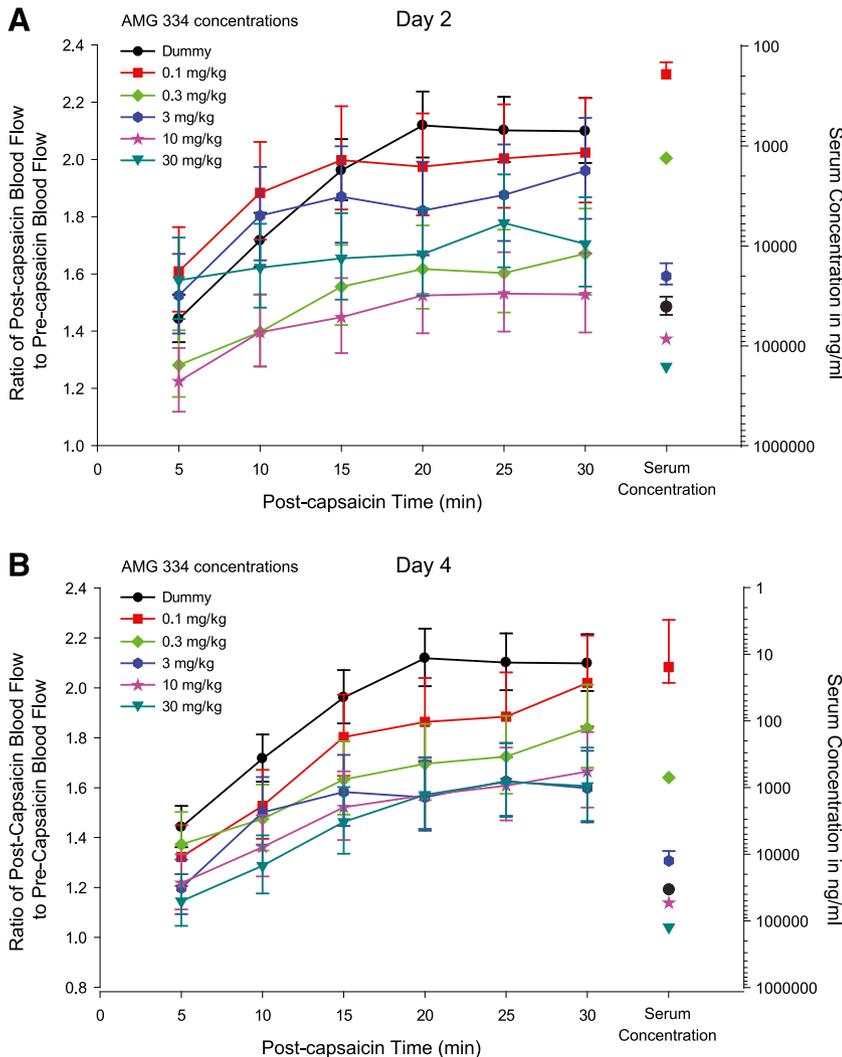


Fig. 5. Dose-dependent reduction of capsaicin-induced increase in local DBF, as measured by laser Doppler imaging, by AMG 334 on day 2 after i.v. administration in cynos. The increase in DBF from local application of capsaicin was quantified by laser Doppler imaging as flux. The left y-axis shows the least-square geometric mean \pm S.E.M. of the flux measured every 5 minutes after application of 1 mg of topical capsaicin and normalized by the pre-capsaicin flux measurement. The right y-axis shows the mean serum concentration (in nanograms per milliliter) immediately after capsaicin flux measurement. Cynos were dosed with AMG 334 (0.1–30 mg/kg; $n = 6$) or α DNP (4 mg/kg; $n = 8$ on day 2, $n = 2$ on day 4). Dose dependency was evaluated using a Jonckheere-Terpstra trend test, with the endpoints adjusted by the pre-capsaicin flux and the capsaicin-induced increase in flux before administration of AMG 334 (day 0). (A) When administered i.v. 48 hours before testing (day 2), AMG 334 produced a significant dose-dependent reduction in capsaicin-induced increase in DBF ($P < 0.05$; one-sided) at each of the 5-minute measurement time points post-capsaicin application from minutes 10 to 30. (B) When administered i.v. 96 hours before testing (day 4), AMG 334 produced a significant dose-dependent reduction in capsaicin-induced increase in DBF ($P < 0.05$; one-sided) at each of the 5-minute measurement time points post-capsaicin application from minutes 5 to 10 and 20 to 30.

receptors compared with dog, rabbit, and rat receptors. It has been previously shown that RAMP1 is responsible for the high-affinity binding of the BIBN4096BS class of antagonists (Mallee et al., 2002). These conclusions were supported by the results of *in vitro*, transient, mixed-species cotransfections of CLR and RAMP1 and by MK-0974 (Salvatore et al., 2008). It is predicted that AMG 334 binds to the CGRP receptor in a manner similar to that of the small-molecule CGRP-receptor antagonists, and RAMP1 governs species selectivity.

The use of capsaicin-induced increase in DBF in NHPs as a translatable pharmacodynamic model for the advancement of small-molecule CGRP antagonists has successfully bridged the path from NHPs to the clinic. Telcagepant significantly and dose-dependently reduced the capsaicin-induced increase in DBF, with an *in vitro* IC₅₀ of 2.2 nM and an *in vivo* IC₅₀ of 127 nM total or 5 nM unbound (Salvatore et al., 2008; Tfelt-Hansen and Olesen, 2011). These results indicate that a half-maximal *in vivo* effect is achieved at unbound plasma concentrations that are approximately twofold in excess of the *in vitro* IC₅₀. When similarly evaluated in humans, telcagepant reduced the capsaicin-induced effect at an IC₅₀ of 101 nM total or 4 nM unbound (Sinclair et al., 2010), a similar approximately 2-fold in excess of the *in vitro* IC₅₀, supporting the clinical translatability of this pharmacodynamic model. This was also true when evaluating the near-maximal effect in NHP relative to humans. The *in vivo* IC₉₀ in rhesus monkeys was 994 nM total or 40 nM unbound, whereas the *in vivo* IC₉₀ in man was 909 nM total or 36 nM unbound, both of which are in the approximate range of 16- to 18-fold in excess of the *in vitro* IC₅₀.

For AMG 334, the minimum significant dose to reduce the capsaicin-induced increase in DBF was 0.3 mg/kg on day 2, but not on day 4. On day 2, immediately after DBF measurements, the resulting plasma concentration for cynos dosed with 0.3 mg/kg was 1320 ng/ml or 8.8 nM, which was 4-fold in excess of the *in vitro* IC₅₀ (using no estimate of protein binding for an antibody). On day 4, the resulting plasma concentration for these same cynos was 707 ng/ml or 4.7 nM, which was approximately 2-fold in excess of the *in vitro* IC₅₀. On day 4, AMG 334 produced a maximal response at doses of 3 mg/kg and higher. The resulting plasma concentration for cynos dosed with 3 mg/kg on day 4 was 12,500 ng/ml or 83 nM, which was approximately 36-fold in excess of the *in vitro* IC₅₀. This relative similarity in the plasma concentration in excess of the *in vitro* IC₅₀ between telcagepant and AMG 334 in NHPs and the proven translatability of telcagepant from NHPs to humans suggests similar clinical translatability of AMG 334. We expect that AMG 334 will significantly reduce capsaicin-induced increase in DBF in humans at a dose that yields unbound plasma concentrations around 4-fold in excess of the *in vitro* IC₅₀ and a maximal effect at a dose that yields unbound plasma concentrations around 36-fold in excess of the *in vitro* IC₅₀.

In addition to high potency and superior selectivity, which may provide the benefit of fewer off-target side effects, monoclonal antibodies provide advantages that make them better suited for preventive treatment compared with small molecules. The half-life of small molecules typically ranges in hours, which is shorter in duration compared with the half-life of monoclonal antibodies, which typically ranges from days to weeks. The prolonged plasma half-life of antibodies allows longer dosing intervals ranging from every 2 weeks to

monthly. Longer dosing intervals are important for preventive treatments because they may be more convenient, which could improve adherence and persistence (Gerber, 2008; Hansel et al., 2010). In addition, unlike small molecules, monoclonal antibodies are not eliminated through hepatic, renal, or biliary processes (Zhou and Mascelli, 2011) and are associated with a significantly reduced risk of drug-to-drug interactions (Gerber, 2008). Because monoclonal antibodies are not degraded by the liver, hepatotoxicity is not anticipated, and problems of elevated liver enzymes as reported with the small-molecule CGRP-receptor antagonists MK-0974 and MK-3207 (Silberstein, 2015) are less of a concern.

Recently, three monoclonal antibodies against CGRP, the ligand of the CGRP receptor, were reported to be efficacious in preventive treatment in patients with episodic migraine (between 4 and 14 migraine headache days per month) or chronic migraine (at least 15 migraine headache days per month). LY2951742, ALD403, and LBR-101 (TEV-48125), all humanized monoclonal antibodies against CGRP, have reported efficacy in both episodic and chronic migraine studies (BusinessWire (2015) Teva's TEV-48125 meets primary and Secondary endpoints in episodic Migraine Study, demonstrating treatment concept after a single dose; and Teva announces positive results for TEV-48125 in phase IIb chronic migraine study meeting primary and secondary endpoints (Dodick et al., 2014a,b). Monthly subcutaneous dosing of AMG 334 is also being evaluated in phase 2 and phase 3 clinical trials. AMG 334 is unique among the novel CGRP monoclonal antibodies for migraine prevention in that it is a fully human monoclonal antibody that targets the CGRP receptor rather than the CGRP ligand.

In conclusion, AMG 334 potently inhibited the binding of [¹²⁵I]-CGRP to the human CGRP receptor, potently antagonized CGRP in a functional assay, and showed species and receptor-family specificity and pharmacodynamic activity. These results provide proof-of-concept that a monoclonal antibody to the CGRP receptor effectively blocks the CGRP signaling pathway and could therefore exhibit therapeutic utility in migraine.

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Wrote or contributed to the writing of the manuscript: Lehto, Shi, Smith, Wild, Xu, Zhu.

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