Translational Pharmacodynamics of Calcitonin Gene-Related Peptide Monoclonal Antibody LY2951742 in a Capsaicin-Induced Dermal Blood Flow Model

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ABSTRACT
LY2951742, a monoclonal antibody targeting calcitonin gene-related peptide (CGRP), is being developed for migraine prevention and osteoarthritis pain. To support the clinical development of LY2951742, capsaicin-induced dermal blood flow (DBF) was used as a target engagement biomarker to assess CGRP activity in nonhuman primates and healthy volunteers. Inhibition of capsaicin-induced DBF in nonhuman primates, measured with laser Doppler imaging, was dose dependent and sustained for at least 29 days after a single intravenous injection of the CGRP antibody. This information was used to generate a pharmacokinetic/pharmacodynamic model, which correctly predicted inhibition of capsaicin-induced DBF in humans starting at a single subcutaneous 5-mg dose. As expected, the degree of inhibition in capsaicin-induced DBF increased with higher LY2951742 plasma concentrations. Utilization of this pharmacodynamic biomarker with pharmacokinetic data collected in phase I studies provided the dose-response relationship that assisted in dose selection for the phase II clinical development of LY2951742.

Introduction
The neuropeptide calcitonin gene-related peptide (CGRP) is a potent vasodilator (Brain et al., 1985), but also has well established roles in neurogenic inflammation and nociception (Hirsch et al., 2013). CGRP is widely expressed in the central and peripheral nervous system and is able to facilitate the production and secretion of numerous proinflammatory mediators that lead to hyperemia, edema, and pain in inflamed tissues (Cady et al., 2011). This peptide can not only have direct excitatory effects on nociceptive neurons, leading to sensitization or activation of neurons in pain signaling pathways, but can also facilitate the effects of other pain transmitters, including glutamate and substance P (Ma et al., 2010). CGRP lowers the activation thresholds and increases excitability in cultured dorsal root ganglia neurons (Natura et al., 2005) and depolarizes sensory neurons in culture (Segond von Banchet et al., 2002), suggesting that CGRP can drive maladaptive processes in peripheral nerves, which induce peripheral sensitization and ultimately pain.

Over the last decade, the importance of CGRP in the trigeminovascular system as a target for migraine treatment has been established (Silberstein, 2013). The proof of principle was provided by BIBN4096BS (olcegepant), the first CGRP receptor (CGRP-R) antagonist that showed therapeutic efficacy in acute migraine treatment with intravenous administration (Olesen et al., 2004). Subsequent clinical trials with the orally available small-molecule CGRP-receptor antagonists MK-0974 (telcagepant) (Ho et al., 2008) and MK-3207 (Hewitt et al., 2011) confirmed efficacy, but these compounds were discontinued because of observed liver toxicity, likely due to off-target binding (Bigal and Walter, 2014). Due to their inherent target specificity, antibodies targeting CGRP or its receptor would most likely avoid such off-target liver toxicity. In addition, antibodies generally display a long pharmacokinetic (PK) profile, making an antibody potentially more suitable for the treatment of chronic conditions.

LY2951742 (Benschop et al., 2007) is a humanized monoclonal antibody that selectively binds and neutralizes CGRP and has been identified for clinical development in migraine prophylaxis (Dodick et al., 2014) and osteoarthritis pain (Benschop et al., 2014). LY2951742 binds both α- and β-CGRP with approximately equal affinity. This paper reports on the translational behavior of LY2951742 in a pharmacodynamic (PD) model of capsaicin-induced dermal blood flow (DBF) in nonhuman primates (NHPs) and healthy human volunteers. In this PD model, the topical application of capsaicin on the

ABBREVIATIONS: ANOVA, analysis of variance; BBB, blood-brain barrier; CGRP, calcitonin gene-related peptide; CGRP-R, calcitonin gene-related peptide receptor; CI, confidence interval; DBF, dermal blood flow; LDI, laser Doppler imaging; NHP, nonhuman primate; PD, pharmacodynamic(s); PK, pharmacokinetic(s).
skin activates transient receptor potential vanilloid type 1 receptors on Aδ- and C-fiber nociceptors (Caterina et al., 1997), which results in the local release of vasoactive substances, including CGRP. The capsaicin-induced DBF change can be quantified using laser Doppler imaging (LDI), and CGRP was identified as the key mediator involved in this model (Van der Schueren et al., 2008). This neurogenic inflammation model, therefore, can be used to evaluate the target engagement of compounds that inhibit the CGBP pathway both preclinically (Salvatore et al., 2008; Benschop et al., 2014) and in clinical development (Salvatore et al., 2010; Sinclair et al., 2010).

Here, we describe the development of a PK/PD model in NHPs to predict the therapeutic doses for the anti-CGRP antibody LY2951742 in human clinical trials. The model was developed in NHP using two closely related antibodies to CGRP, testing their ability to neutralize capsaicin-induced DBF. Ultimately, the objective was to assess the interspecies translational behavior of LY2951742 in the DBF model and demonstrate neutralization of CGRP with this antibody in humans. This translational biomarker model was used to predict efficacious doses of LY2951742 in early clinical development for prophylactic migraine treatment and osteoarthritis pain.

Materials and Methods

NHP Studies

To generate antibodies specific to human CGRP, BALB/c mice were immunized with human CGRP conjugated to ovalbumin. Human IgG4 antibodies were generated as previously described (Benschop et al., 2014). In these studies, two related and equally potent [based on inhibition of CGRP-induced cAMP production in SK-N-MC cells in vitro (Benschop et al., 2014)] CGRP neutralizing antibodies (LSN2915644 and LY2951742) were used.

Study Population. Animal studies were performed under protocols approved by the Eli Lilly Institutional Animal Care and Use Committee.

Cynomolgus NHPs were enrolled in the study based on prescreening for capsaicin responsiveness. NHPs that exhibited a ≥50% increase in blood flow over baseline with 2-mg topical capsaicin treatment and stable physiology during the imaging period were included in a study with either LSN2915644 or LY2951742.

The study population included healthy, antibody-naive cynomolgus NHP males weighing ~3–4 kg.

Dose Administration. NHPs received vehicle, 0.1, 1, or 5 mg/kg (LSN2915644) or 5 mg/kg (LY2951742) of the antibody administered intravenously 1 day prior to the day 1 LDI experiment. Animals that received 0.1 mg/kg LSN2915644 showed lack of DBF inhibition by day 15 and were given a follow-up dose of 15 mg/kg LSN2915644 and re-evaluated.

Pharmacodynamic Sampling. LDI was performed on days 1, 15, and/or 29 post-treatment with anti-CGRP antibodies. Animals were fasted overnight prior to each capsaicin challenge. On the day of the experiment, the NHP was anesthetized with 1% isoflurane for approximately 30 minutes prior to scanning. The NHP was placed in a quiet, temperature-controlled room supine on a warm small surgical blanket, and the shaved arm was placed on a heating pad under the laser head. Three neoprene O-rings were placed on the NHP forearm approximately 1 cm apart. During a 30-minute stabilization period, preliminary scans were obtained to confirm the correct positioning of the O-rings. Once baseline temperature (approximately 37°C) was stabilized, a baseline scan was collected. After the baseline scan was completed, 20 μL of capsaicin solution (50 mg of capsaicin in a solution of 170 μL ethanol, 80 μL Tween 20, and 250 μL purified H2O) was applied to each O-ring. Scanning continued every 5 minutes for an additional 25 minutes [methods adapted from earlier published procedures (Hershey et al., 2005)]. Study operators and data analysts were blinded to the treatments (CGRP antibody or vehicle).

Pharmacokinetic Sampling. The PK profile of the CGRP antibodies LY2951742 and LSN2915644 were characterized in healthy male cynomolgus NHPs (n = 2–3) following a single intravenous bolus injection of 2 mg/kg. Plasma concentrations of the antibodies were determined at 1, 3, 6, 12, 24, 48, 96, 120, 168, 240, 336, 504, and 672 hours postdose by a sandwich enzyme-linked immunosorbent assay. The mean PK parameter estimates were obtained by fitting the observed mean plasma concentration-time profile to a two-compartment model using WinNonLin.

Analysis and Statistics. LDI repeat scans were analyzed using version 5.2 Moor software (Moor Instruments, Axminster, UK) for region of interest analysis and Microsoft Excel worksheets for averaging the signal from the regions of interest at a given time point. Changes in DBF were reported as a percentage change from baseline. Analyzed data were entered into GraphPad Prism 4 (La Jolla, CA) for graphing, and a repeated measurement mixed-effect model in SAS 9.1 (SAS Institute Inc., Cary, NC) was used for statistical analysis. Data are expressed as mean ± S.D.

Human Studies

Study Design and Population. After approval by the ethics committee of the University Hospitals of Leuven (Leuven, Belgium), written informed consent was obtained from all subjects during a screening visit.

This study was a single-site, double-blind, placebo-controlled, single- and multiple-dose escalation study of LY2951742 in healthy volunteers. The study population included healthy nonsmoking white males, 18–55 years old, with a body mass index ≥ 19 kg/m². Six cohorts of nine subjects [two receiving placebo (vehicle) and seven receiving LY2951742] each were administered a single dose, and a single cohort received LY2951742 administered every other week for a total of four administrations. Each subject and cohort was evaluated for safety, PK, and PD.

Dose Administration. Subjects in the single-dose cohorts received subcutaneous doses of LY2951742 of 1, 5, 25, 75, 200, or 600 mg or placebo. No subject participated in more than one cohort. A maximum volume of 1.5 mL per injection was allowed. The placebo solution/injection was indistinguishable in appearance and volume from the LY2951742 solution/injection. One multidose group was included (n = 7). Subjects received a total of four injections of 150 mg s.c. every other week, resulting in a total dose of 600 mg.

Pharmacodynamic Sampling. Capsaicin-induced DBF was assessed after topical application of capsaicin (1000 μg capsaicin dissolved in a 3:3:4 mixture of ethanol 100%, Tween 20, and distilled water) or vehicle using LDI according to earlier published procedures (Van der Schueren et al., 2007, 2008).

In summary, all measurements for the assessment of the capsaicin-induced DBF response were performed while the subjects were resting in a supine position on a bed in a quiet, temperature-controlled room (22 ± 1°C). During each visit (screening and study periods), three rubber O-rings were placed on the volar surface of the forearm. After placement of the O-rings, an LDI system (HR-LDPI, Periscan PIMII; Perimed, Järfälla, Sweden) was used to obtain baseline scans of the DBF of the areas defined by the rings. In subsequent evaluations, these O-rings served as reservoirs to contain the observed mean plasma concentration-time profile to a two-compartment model using WinNonLin.

The change from baseline measurements at two capsaicin rings was averaged for use in PK/PD evaluations after subtraction of the measurement in the vehicle O-ring.

Analysis and Statistics. LDI repeat scans were analyzed using version 5.2 Moor software (Moor Instruments, Axminster, UK) for region of interest analysis and Microsoft Excel worksheets for averaging the signal from the regions of interest at a given time point. Changes in DBF were reported as a percentage change from baseline. Analyzed data were entered into GraphPad Prism 4 (La Jolla, CA) for graphing, and a repeated measurement mixed-effect model in SAS 9.1 (SAS Institute Inc., Cary, NC) was used for statistical analysis. Data are expressed as mean ± S.D.
The dermal capsaicin challenge was performed as part of the screening procedures to exclude nonresponders, i.e., <100% DBF increase from baseline, with baseline defined as DBF before capsaicin application, and to establish a pre-LY2951742 DBF evaluation.

For all single-dose cohorts, the dermal capsaicin challenge was performed during the screening period, 48–56 hours after dosing on day 3, and on days 14 (±2 days), 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, 113, 141, and 176 (±2 days) or during the visit closest in time to the days that DBF was planned per protocol. The serum concentration of LY2951742 was measured in samples collected at the same time points as LDI by a validated enzyme-linked immunosorbent assay method.

Analysis and Statistics. The statistical analyses were performed using SAS version 9.2. The mean change from predose by dose group and time, along with 95% confidence interval (CI) on the difference between LY2951742 and placebo, was calculated. An analysis of variance (ANOVA) model was used to analyze the DBF changes induced by capsaicin without taking into account the baseline data. The ANOVA model included the subject (as a random effect), time, and dose group. A two-sided 95% CI for the difference (LY2951742-placebo) in log-scale capsaicin response was computed from the ANOVA. The CI was back-transformed to obtain a geometric mean and corresponding CI for the mean ratio for the fold difference from placebo in the capsaicin response. The resulting geometric mean ratio and corresponding CIs were transformed to the percentage difference from placebo using the following formula: percentage difference from placebo = [1−(fold difference from placebo)]*100. These estimates were calculated for each time point and LY2951742 dose group.

A repeated measures mixed model was fitted for change from the predose of DBF (vehicle corrected) with time, dose group, and time*dose group as fixed effects, percentage change DBF at predose as the covariate, and subject within time as the random effect. Change from the predose of DBF with correction for baseline is computed as ∑[(w1*y1+w2*y2)/(w1+w2)]−(w1*x1+w2*x2)/(w1+w2)), DBF at time = 0 minutes is a1, a2 (w1, w2) for capsaicin sites and b(x) for the vehicle site at predose (postdose time points). DBF at time = 30 minutes is c1, c2 (y1, y2) for the capsaicin sites and d(x) for the vehicle site at predose (postdose time points). From this analysis, the latest time point at which the drug was effective with respect to LDI results was determined for each dose group separately.

Results

PK and Capsaicin-Induced DBF in the NHP. PK parameter estimates of LSN2915644 and LY2951742 after a single intravenous administration of 2 mg/kg in NHP were
obtained by fitting the mean plasma concentration-time profile to a two-compartment model using WinNonlin (Fig. 1A).

Repeatability of capsaicin-induced DBF increase was confirmed in the control group (Fig. 1B), allowing us to compare DBF after 29 days relative to each animals’ own capsaicin-induced DBF response at preantibody dosing.

The first NHP LDI study used LSN2915644 to test the ability of a CGRP-neutralizing antibody to block the capsaicin-induced enhancement of DBF. LSN2915644 is a closely related variant of LY2951742 with similar potency to LY2951742. The effect of LSN2915644 was evaluated at concentrations of 0.1, 1, or 5 mg/kg, with intravenous dosing in the NHP. The DBF percentage change from baseline was calculated for the 25-minute scan after capsaicin application and determined before and 1 and 15 days post-LSN2915644 dosing (Fig. 1C). We observed a dose-dependent inhibition of DBF with the CGRP antibody. DBF was significantly inhibited at 5 mg/kg at both time points, whereas no significant inhibition was observed in the two lowest dose groups (0.1 and 1 mg/kg) (Fig. 1C).

Inhibition of capsaicin-induced DBF was determined in a sub-group of animals (n=4) from the 5-mg/kg dose group and was observed for an additional 2 weeks. Animals showed a sustained (70%) inhibition of the capsaicin-induced enhancement in DBF (data not shown). Four animals from the nonneficiacious 0.1-mg/kg group were additionally dosed with 15 mg/kg LSN2915644, and LDI was tested. The results showed rapid and significant inhibition of capsaicin-induced DBF (Fig. 1D), similar to the 5-mg/kg dose group, demonstrating that capsaicin-induced DBF could be inhibited by a CGRP antibody in these animals and that 0.1 mg/kg was an insufficient dose to significantly inhibit the effect of capsaicin. Based on these results, the ability of LY2951742 to inhibit DBF was determined at a single dose level (5 mg/kg) using the same study design. The efficacy of LY2951742 at each time point was comparable to that observed with LSN2915644 (Fig. 2A).

**PK/PD Modeling of Capsaicin-Induced DBF in the NHP.** Data from the DBF model in the two NHP studies were used to develop a PK/PD relationship predicting the efficacy of LY2951742 in inhibiting capsaicin-induced changes in DBF in humans. No significant difference between the two studies was found in terms of PK or PD of the two antibodies tested overall or at each time point. Therefore, PK and PD data from both studies were combined and fitted simultaneously to a linear PK/PD model using NONMEM. Population PK/PD parameters for the NHP DBF data are summarized in Table 1. The relationship between effect-site concentrations of LSN2915644 and LY2951742 and percentage change in blood flow from baseline at 25 minutes postcapsaicin was described by an inhibitory $E_{\text{max}}$ model (Fig. 2; Table 1).

**Projection of Potential Efficacious Dose Level in Humans.** A 5 mg/kg i.v. dose in NHP resulted in a peak plasma concentration of approximately 150,000 ng/ml. PD effects were still observed at 29 days after dose administration. With an apparent plasma half-life of 7.6 days in NHP, the estimated plasma LY2951742 concentration at 29 days after infusion (i.e., after about four half-lives) is approximately 10,000 ng/ml. Modeling of these data from the PD study in NHPs suggest an EC$_{50}$ of 5560 ng/ml. The predicted central distribution volume in humans is 32.6 ml/kg, which translates into a 2-l volume in a 60-kg person. Calculation of the dose in humans to achieve an EC$_{50}$ plasma concentration is therefore 11.1 mg i.v. or 26 mg s.c. (assuming 40% bioavailability).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± S.E.</th>
<th>SEE</th>
</tr>
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<tbody>
<tr>
<td>CL (ml/h per kg)</td>
<td>0.253 ± 0.0171</td>
<td>6.76</td>
</tr>
<tr>
<td>V (ml/kg)</td>
<td>47.1 ± 2.04</td>
<td>4.33</td>
</tr>
<tr>
<td>$E_0$ (%)</td>
<td>76.0 ± 6.11</td>
<td>8.04</td>
</tr>
<tr>
<td>$E_{\text{max}}$ (%)</td>
<td>61.1 ± 10.1</td>
<td>16.5</td>
</tr>
<tr>
<td>EC$_{50}$ (ng/ml)</td>
<td>5560 ± 2670</td>
<td>48.0</td>
</tr>
<tr>
<td>$k_{\text{eq}}$ (h$^{-1}$)</td>
<td>0.00533 ± 0.00129</td>
<td>24.2</td>
</tr>
<tr>
<td>IV in CL</td>
<td>0.0746 ± 0.0190</td>
<td>25.5</td>
</tr>
<tr>
<td>IV in $E_0$</td>
<td>0.0125 ± 0.00599</td>
<td>47.9</td>
</tr>
<tr>
<td>Residual error for PK</td>
<td>0.0679 ± 0.0109</td>
<td>16.1</td>
</tr>
<tr>
<td>Residual error for PD</td>
<td>0.305 ± 0.0996</td>
<td>32.7</td>
</tr>
</tbody>
</table>

CL, clearance; IV, interindividual variability in the parameters; SEE, standard error of the estimate.

**Capsaicin-Induced DBF in Humans.** Demographic characteristics of participating subjects did not differ between the placebo (n = 12) or LY2951742 treatment groups.
groups (six cohorts of seven subjects). The 54 subjects included white males with a median age of 27 years (range, 18–54 years) and a median body mass index of 24.4 kg/m² (range, 19.5–34.2 kg/m²).

Mean profiles of the absolute changes in capsaicin-induced DBF for each dose group over time are displayed in Fig. 3A. Compared with placebo, the mean relative change in DBF induced by capsaicin was significantly reduced (Table 2) in the 75-, 200-, and 600-mg dose cohorts at all postdose time points (days 3–42). In addition, significant differences from placebo were observed in the 25-mg dose cohort on days 14, 28, and 42 and in the 5-mg dose cohort on days 28 and 42. Subjects receiving a single dose of 1 mg LY295174 did not differ from placebo at any time point. Figure 3B displays the relationship between capsaicin-induced DBF and LY295174 serum concentration after single-dose administration. Higher exposure to LY295174 corresponds with greater inhibition of capsaicin-induced DBF response.

Notable inhibition of capsaicin-induced DBF was observed on day 28, when the percentage difference from placebo averaged 5.9% (95% CI, −152.4; 64.9) after 1 mg, 55.7% (95% CI, −18.9; 83.5) after 5 mg, 67.4% (95% CI, 12.6; 87.8) after 25 mg, 77.2% (95% CI, 38.9; 91.5) after 75 mg, 82.8% (95% CI, 53.2; 93.6) after 200 mg, and 77.9% (95% CI, 40.8; 91.8) after 600 mg (Fig. 4). Combined, Figs. 3 and 4 illustrate the dose-response relationship between LY295174 and inhibition of DBF response.

One cohort received four doses of 150 mg LY295174 every other week, and DBF responses were assessed at regular intervals for a total of 176 days (Fig. 5). An increase in LY295174 serum concentration was observed over the 6-week dosing period, which was accompanied by a rapid and sustained suppression of capsaicin-induced DBF. Analogous to the single dose results, multiple-dose exposure to LY295174 inversely correlated with DBF response from day 14 up to at least 176 days after first administration.

**Discussion**

The PD behaviors of monoclonal antibodies targeting CGRP in NHPs and healthy human volunteers were detailed and modeled to predict efficacious doses in the clinic. A capsaicin-induced DBF PD assay was applied to assess the efficacy with which antibodies can neutralize endogenous CGRP released in response to the local application of capsaicin. This DBF assay leverages the translatability of laser Doppler imaging from preclinical to clinical measures. Long-term inhibition of CGRP by both monoclonal antibodies (LSN2915644 and LY295174) was observed in NHPs. Subsequently, these

### TABLE 2

Comparison between LY295174 and placebo in the percentage change from baseline in DBF, with correction for baseline by time and dose group

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Placebo</th>
<th>LY295174</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td>352.638 (94.100)</td>
<td>402.437 (57.995)</td>
</tr>
<tr>
<td>Mean (S.D.)</td>
<td>357.236 (103.165)</td>
<td>341.696 (106.793)</td>
</tr>
<tr>
<td>Day 3 Mean (S.D.)</td>
<td>339.298 (118.864)</td>
<td>345.932 (102.355)</td>
</tr>
<tr>
<td>P value</td>
<td>0.701</td>
<td>0.3797</td>
</tr>
<tr>
<td>Day 14 Mean (S.D.)</td>
<td>319.094 (119.201)</td>
<td>359.752 (112.561)</td>
</tr>
<tr>
<td>P value</td>
<td>0.7879</td>
<td>0.0994</td>
</tr>
<tr>
<td>Day 28 Mean (S.D.)</td>
<td>359.224 (102.101)</td>
<td>336.811 (132.196)</td>
</tr>
<tr>
<td>P value</td>
<td>0.2871</td>
<td>0.0063</td>
</tr>
<tr>
<td>Day 42 Mean (S.D.)</td>
<td>350.035 (107.829)</td>
<td>344.652 (108.296)</td>
</tr>
<tr>
<td>P value</td>
<td>0.36</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Comparison between LY295174 and placebo in the percentage change from baseline in DBF, with correction for baseline by time and dose group

Values presented are percentages. Mixed model repeated measures for change from predose in percentage change DBF induced by capsaicin, with time, dose group, and time*dose group as the fixed effects, percentage change DBF at predose as the covariate, and subject within time as the random effect.
LY2951742 antibodies bind CGRP with high affinity (10 and 2010; Sinclair et al., 2010) settings. Both LSN2915644 and 2005; Salvatore et al., 2008) and clinical (Salvatore et al., 2014). A similar method has been used by others to assess the receptor in the periphery in both preclinical (Hershey et al., 2014). A similar method has been used by others to assess the efficiency of LY2951742 in animals for a prolonged period of time. We have previously reported the inhibition of capsaicin-neutralization antibody in NHP. The results obtained with two different antibodies to CGRP (LSN2915644 and LY2951742) clearly demonstrated that both antibodies can effectively neutralize CGRP in vivo in animals for a prolonged period of time. We have previously reported the inhibition of capsaicin-induced DBF in the rat using LY2951742 (Benschop et al., 2014). A similar method has been used by others to assess the target engagement of small molecule inhibitors of the CGRP receptor in the periphery in both preclinical (Hershey et al., 2005; Salvatore et al., 2008) and clinical (Salvatore et al., 2010; Sinclair et al., 2010) settings. Both LSN2915644 and LY2951742 antibodies bind CGRP with high affinity (10 and 31 pM, respectively; R. J. Benschop, unpublished data, and Benschop et al., 2014). In dose-escalation experiments with single intravenous administration, LSN2915644 displayed clear dose-dependent inhibition of capsaicin-induced DBF. Additionally, giving a high (15 mg/kg) dose to animals previously dosed with a no-effect dose of 0.1 mg/kg resulted in a significant decrease in capsaicin-induced DBF, confirming a maximal effect was reached at 5 mg/kg. After both LSN2915644 and LY2951742 exhibited comparable preclinical efficacy in this PD assay, LY2951742 was taken forward for clinical development. The aggregate preclinical results were used to inform the PK/PD model, which allowed a clinical dose projection for LY2951742.

Mediation of CGRP, CGRP-R, and transient receptor potential receptors has been investigated for the treatment of pain indications, such as migraine. The small molecule CGRP-R antagonists olecegapant (BIBN4096BS) and telcagepant (MK-0974) showed clear efficacy for acute migraine treatment (Olesen et al., 2004; Ho et al., 2008; Hewitt et al., 2011). More recently, antibodies have been reported targeting either CGRP or the CGRP receptor (Bigal and Walter, 2014). However, LY2951742 is the first antibody providing evidence in a phase II trial to be safe and efficacious in migraine prevention (Dodick et al., 2014). Although the exact mechanistic link between CGRP, CGRP-R, and migraine headache remains unclear, evidence for CGRP involvement in migraine pathophysiology is growing and CGRP remains a key target for migraine treatment.

Capsaicin-induced vasodilatation monitored by LDI was used in our study as a biologically relevant response to neutralization of CGRP after a single injection with a CGRP-neutralizing antibody in NHP. The results obtained with two different antibodies to CGRP (LSN2915644 and LY2951742) clearly demonstrated that both antibodies can effectively neutralize CGRP in vivo in animals for a prolonged period of time. We have previously reported the inhibition of capsaicin-induced DBF in the rat using LY2951742 (Benschop et al., 2014). A similar method has been used by others to assess the target engagement of small molecule inhibitors of the CGRP receptor in the periphery in both preclinical (Hershey et al., 2005; Salvatore et al., 2008) and clinical (Salvatore et al., 2010; Sinclair et al., 2010) settings. Both LSN2915644 and LY2951742 antibodies bind CGRP with high affinity (10 and 31 pM, respectively; R. J. Benschop, unpublished data, and Benschop et al., 2014). In dose-escalation experiments with single intravenous administration, LSN2915644 displayed clear dose-dependent inhibition of capsaicin-induced DBF. Additionally, giving a high (15 mg/kg) dose to animals previously dosed with a no-effect dose of 0.1 mg/kg resulted in a significant decrease in capsaicin-induced DBF, confirming a maximal effect was reached at 5 mg/kg. After both LSN2915644 and LY2951742 exhibited comparable preclinical efficacy in this PD assay, LY2951742 was taken forward for clinical development. The aggregate preclinical results were used to inform the PK/PD model, which allowed a clinical dose projection for LY2951742.

Given that the CGRP sequence between humans and cynomolgus NHP is identical (R. J. Benschop, unpublished data), in vivo NHP pharmacology data are relevant for predicting in vivo human activity. On the basis of allometric scaling and assuming 100% bioavailability, the plasma EC50 would be achieved by an 11.1-mg dose in humans, and 1 mg was chosen as the starting dose for the phase 1 clinical trial. Interspecies comparison of the PD assay with LY2951742 displayed comparable efficacy. Maximal PD effect of the neutralizing antibody observed in both species could not completely block DBF increase in response to capsaicin. These data with LY2951742 are in agreement with other human studies using CGRP-R antagonists (CGRP8-37, telcagepant, and MK-3207), which also did not completely block the DBF response to capsaicin. In the case of CGRP8-37, it was suggested that this is due to its relatively lower affinity. The small molecules previously tested in this assay (MK-3207 and telcagepant) displayed higher affinity to CGRP-R than CGRP8-37, but still did not block the DBF response completely (Sinclair et al., 2010; Li et al., 2014). Mediators, such as nitric oxide, substance P, and prostaglandins, were previously excluded from playing a significant role in the capsaicin assay (Van der Schueren et al., 2008). Therefore, it might be that a small residual part of the DBF response is mediated by
histamine or other vasodilators. Nevertheless, LY2951742 exhibits comparable maximal inhibition of capsaicin-induced DBF as telcagepant, a CGRP-R antagonist that showed efficacy in phase 3 migraine clinical trials at doses that inhibited capsaicin-induced DBF (Ho et al., 2008; Sinclair et al., 2010).

In humans, LY2951742 blocked the capsaicin-induced increase in DBF. Comparison of subjects dosed with placebo to those dosed with LY2951742 indicates an exposure-response relationship between LY2951742 and DBF inhibition. As can be seen in Fig. 3B, the 4°150 mg dosing scheme provided the most robust inhibition of capsaicin-induced DBF. This dosing scheme was used for phase 2 testing. Lower doses are being tested in current clinical trials.

At doses of 5 mg and greater, PD effects were observed 3 days after LY2951742 administration and lasted until at least day 42, which was determined as the last day of PD assessment per protocol. As a consequence of the antibody’s long half-life observed across doses, ranging from 25 to 30 days, it is highly plausible that 5 mg of LY2951742 or more could display long-lasting pharmacologic effects for more than 6 weeks. LY2951742 targets the CGRP ligand itself and must immediately capture CGRP, thereby inhibiting initiation and/or progression of the migraine attack. Since antibodies have very limited ability to cross the blood-brain barrier (BBB), this antibody is expected to be primarily active in the periphery (Reuter, 2014). The successful proof-of-concept study with LY2951742 (Dodick et al., 2014) challenges our pathophysiological knowledge on migraine prevention (Reuter, 2014). Recent positron emission tomography studies with telcagepant (Vermeersch et al., 2013) in healthy volunteers and also in migraine patients during a migraine attack suggest limited central penetration in the central nervous system, indicating that CGRP-R antagonists do not necessarily need to penetrate the BBB to be effective in migraine treatment (Reuter, 2014).

This is in agreement with the observation that several parts of the brain involved in pain transmission by the trigeminal nerve appear not to be protected by the BBB and are therefore potential targets of CGRP-R antagonists (Eftekhar et al., 2013). Additionally, should CGRP antibodies prove efficacious for the treatment of other chronic impairments, such as osteoarthritis (Mapp et al., 2012; Hirsch et al., 2013; Benschop et al., 2014) and menopausal flushing (Gupta et al., 2007; Hay and Poyner, 2009), the favorable dosing scheme of antibodies over small molecules could increase their therapeutic value. The first positive results from clinical proof-of-concept studies in migraine prevention with LY2951742 and ALD-403 were presented at the 2014 American Academy of Neurology conference, and additional clinical trials with LY2951742 (Bigal and Walter, 2014) and other antibodies to CGRP (LBR-101) or CGRP-R (AMG334) are underway.

In conclusion, the PD effects of two monoclonal antibodies against CGRP (LSN2915644 and LY2951742) were tested in NHPs with a DBF assay. This biomarker model demonstrated that both antibodies potently and durably inhibit the CGRP pathway. Although there are some differences in the preclinical and clinical PD assay methods that warrant caution with direct data comparison, capsaicin-induced vasodilatation monitored by LDI proves to be a useful translatable tool. A PK/PD model was set up, and translational dose calculations were performed to predict actual doses of LY2951742 that exhibited PD effects in a human clinical trial. The PK/PD relationship was scalable and predictable from NHP studies to human clinical trials. Utilization of this PD model provided a means for understanding a PK and PD relationship that assisted in the clinical development of LY2951742 for migraine prevention and possibly additional indications in the future.

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