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A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Two-Period Crossover Study to Investigate the Safety of CAL-101 in Allergic Rhinitis Subjects and Effects on the Response to Environmental Chamber Allergen Challenge

Investigational Product:	CAL-101
Protocol No.:	101-04
EudraCT Number:	2008-006908-32
Indication studied:	Allergic rhinitis
Study Initiation Date:	26 January 2009
Study Completion Date:	19 March 2009
Sponsor:	Calistoga Pharmaceuticals, Inc. 2101 4th Avenue, Suite 1960 Seattle, WA 98121
Sponsor's Medical Expert:	Albert Yu, MD Chief Medical Officer PPD
Principal Investigator:	Prof. Dr. Friedrich Horak PPD
Date of Report:	03 August 2009

This study was conducted in compliance with the International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP) and applicable laws and regulations

2 SYNOPSIS

Name of Sponsor/Company: Calistoga Pharmaceuticals, Inc.	Individual Study Table Referring to Part Of the Dossier Volume: Page:	(For National Authority Use Only)
Name of Finished Product: Not applicable		
Name of Active Ingredient: CAL-101		

Title of Study: A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Two-Period Crossover Study to Investigate the Safety of CAL-101 in Allergic Rhinitis Subjects and Effects on the Response to Environmental Chamber Allergen Challenge

Investigator: Prof. Dr. Friedrich Horak
Study Center: Allergy Center Vienna West
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Publication Based on the Study: None at the time of report preparation.

Study Period: **Phase of Development:** 1
First subject, first visit: 26 January 2009
Last subject, last visit: 19 March 2009

Objectives:
The primary objectives of the study were:

- to evaluate the safety of multiple-dose oral administration of CAL-101 in allergic rhinitis subjects, and
- to determine the effects of multiple-dose oral administration of CAL-1-1 on total nasal symptom scores following an environmental chamber allergen challenge in allergic rhinitis subjects.

Secondary objectives were:

- to determine the effects of multiple-dose oral administration of CAL-101 on total nonnasal symptom scores following an environmental chamber allergen challenge in allergic rhinitis subjects,
- to determine the effects of multiple-dose oral administration of CAL-101 on total symptom scores following an environmental chamber allergen challenge in allergic rhinitis subjects,

- to determine the effects of multiple-dose oral administration of CAL-101 on nasal airflows following an environmental chamber allergen challenge in allergic rhinitis subjects,
- to determine the effects of multiple-dose oral administration of CAL-101 on nasal secretion weights following an environmental chamber allergen challenge in allergic rhinitis subjects,
- to determine the effects of multiple-dose oral administration of CAL-101 on nasal scraping eosinophils following an environmental chamber allergen challenge in allergic rhinitis subjects, and
- to determine the pharmacokinetic/pharmacodynamic (PK/PD) effects of CAL-101 in allergic rhinitis subjects.

Methodology: This study consisted of six periods: screening to determine eligibility, a washout period (≥7 days) prior to the first treatment period (7 days), a washout period (≥14 days) between treatment periods, the second treatment period (7 days), and a follow-up evaluation ≥7 days after the last dose of study drug. Subjects were randomly assigned to one of two treatment sequences [CAL-101/placebo (CAL/PL) or PL/CAL] in the order in which they qualified for the study. During each treatment period, subjects took 2 capsules of the assigned study drug twice daily at approximately 12-hour intervals.

Environmental chamber allergen challenges were performed at screening (2 hour exposure) and on the 7th day of each treatment period (4 hour exposure beginning 1 hour after the morning dose of study drug) using the Vienna Challenge Chamber (VCC).

Number of Subjects: The study planned to enroll 45 subjects with a history of seasonal allergic rhinitis in order to obtain data for 36 subjects completing both treatment periods. A total of 47 subjects were screened. Of these, 6 subjects were screen failures, and 41 subjects were randomized, with 21 subjects randomized to the CAL/PL sequence and 20 subjects randomized to the PL/CAL sequence. Two subjects did not participate in Period 2. Subject 143 (PL/CAL) was withdrawn after completing Period 1 (PL) due to clinically significant elevations in hepatic enzymes, and Subject 139 (PL/CAL) withdrew from the study before completing Period 1 (PL) due to scheduling problems. All other subjects completed the study according to the protocol.

The safety population consisted of the 41 randomized subjects. The full analysis set (FAS) consisted of 39 subjects who completed the protocol, with 21 randomized to CAL/PL treatment sequence and 18 randomized to PL/CAL treatment sequence.

Diagnosis and Main Criteria for Inclusion: Males and females without childbearing potential who were ≥18 and ≤55 years old and provided written informed consent were eligible for the study if they had a documented history of seasonal allergic rhinitis for at least 2 years, had a positive response to skin prick testing (wheal ≥3 mm larger than the wheal of the negative control) and a positive Radio Allergen Sorbent Test (≥ class 2) for grass pollen in the 12 months prior to screening; had an adequate response to 1500 grass pollen grains/m^3

within 2 hours in the VCC during screening, defined as a total nasal symptom score (TNSS) ≥6; were otherwise healthy, and were asymptomatic (TNSS ≤3 and a score ≤1 for any single nasal symptom prior to the screening allergen challenge), and did not have any other condition that would make the subject unlikely to be able to remain in the VCC for 4 hours.

Subjects were not eligible for the study if they had a history of chronic nasal or upper respiratory tract symptoms or disorders other than allergic rhinitis; history of nonallergic rhinitis, chronic sinusitis or severe asthma as defined by the GINA guidelines, a nasal condition likely to affect the outcome of the study, i.e. nasal septal perforations, nasal polyps, sinus disease, chronic nasal obstruction, or other nasal diseases; a history of respiratory tract infection within 14 days prior to Visit 1, or a medical condition other than allergic rhinitis that could have posed a significant safety risk or was likely to affect the outcome of the study

Test Product, Dose and Mode of Administration, Batch No.:
CAL-101 100 mg administered orally twice daily for 7 days (only the morning dose was administered on Day 7), Lot/Batch no.: B080405, Expiration: September 2009.

Reference Therapy, Dose and Mode of Administration, Batch No.:
Placebo administered orally twice daily for 7 days (only the morning dose was administered on Day 7), Lot/Batch no.: B080405, Expiration: September 2009.

Duration of Treatment:
The duration of each subject's study participation was expected to be at least 42 days and could be longer if the washout periods were longer than the minimum specified in the protocol. Each subject was exposed to CAL-101 for 7 days and placebo for 7 days (only the morning dose was administered on Day 7).

Criteria for Evaluation:
Efficacy:
Efficacy assessments included allergic rhinitis symptoms, nasal airflow, nasal secretion weight, and nasal cytology.

Allergic rhinitis symptoms were recorded according to the study site's standard operating procedures (SOPs). Symptoms were rated on a 4-point scale: 0 (no symptoms), 1 (mild symptoms), 2 (moderate symptoms), 3 (severe symptoms). Nasal symptoms were nasal congestion, nasal rhinorrhea, nasal itching and sneezing attacks. Nonnasal symptoms were itching eyes, tearing eyes, eye redness and itching of the ears and palate. The variables were rated as single symptoms scores and composite symptom scores defined as:

- total nasal symptom score (TNSS): the sum of the 4 nasal symptom scores (range 0 to 12)
- total nonnasal symptom score (TNNSS), i.e., the sum of the 4 nonnasal symptom scores (range 0 to 12)
- total symptom score (TSS), i.e., the sum of the TNSS and the TNNSS (range 0 to 24).

The primary efficacy variable of the study was the TNSS.

Nasal airflow was determined according to the study site's SOPs, using active anterior rhinomanometry at a pressure difference of 150 Pa across the nasal passages (sum of the right and left nostril values). Nasal secretions were collected according to the study site's SOPs, using pre-weighed packets of absorbent paper tissues. Subjects cleared their noses using the tissue provided at baseline and whenever necessary during allergen exposure. Nasal secretions were recorded every 30 minutes. Nasal scraping samples were collected for cytological analysis according to the study site's SOPs. The cytology measures included total number of leukocytes per high power field, number of eosinophils, and percentage of eosinophils.

Pharmacokinetics:
Plasma PK samples were obtained before and 1.5 and 3 hours after the morning dose on Day 1 and before the morning dose and at the end of the VCC challenge on Day 7 of each treatment period. Plasma concentrations were determined using a validated LC-MS/MS assay.

Pharmacodynamics:
Whole blood PD samples were obtained before and 1.5 hours and 3 hours after the morning dose on Day 1 of each treatment period. The percent of CD63+/CCR3+ cells following ex vivo stimulation with grass pollen antigen was determined for each sample from all subjects during the CAL-101 treatment period and a subset of subjects during the placebo treatment period.

Safety:
Adverse events, vital signs, physical examinations, clinical laboratory tests (hematology, clinical chemistry, urinalysis, and pregnancy tests for women of childbearing potential), ECGs, and spirometry.

Statistical Methods:
The primary and secondary endpoints derived from the VCC exposure were summarized using descriptive statistics for each VCC exposure, i.e., period 1 and period 2. For each period, the change from baseline for the primary and secondary variables was summarized for the time weighted average ($AUC_{2\text{-}4h}/2$) after exposure in the VCC. The baseline for these calculations was the last recorded value prior to exposure to allergen in the VCC. Changes from baseline in each efficacy variable were analyzed using the crossover technique for the continuous variables. Treatment comparisons were summarized using the least square means, standard error (SE), and 95% CI from the ANOVA.

The plasma concentration results were summarized by time using descriptive statistics.

For the PD assessment, the percent of CD63+/CCR3+ cells following grass pollen antigen stimulation in pre-dose samples was the baseline, and the CD63+/CCR3+ cells in post-dose samples were expressed as a relative percent of the baseline. A scatter plot of the inhibition

of ex vivo basophil activation and the plasma concentration of CAL-101 obtained at the same time point was generated.

The incidence of treatment-emergent adverse events (TEAEs) was summarized by treatment for all TEAEs, drug-related TEAEs, SAEs, drug-related SAEs, and discontinuations due to TEAEs. The incidence and number of events were summarized by MedDRA system organ class, preferred term, and treatment. Observed values and changes from baseline were summarized by treatment for clinical laboratory tests, spirometry, ECG, and vital signs.

SUMMARY – CONCLUSIONS

The safety population consisted of 41 (100%) male subjects who ranged in age from 20 to 49 years (mean: 27.6 years). Most subjects were Caucasian (90.2%). The median time since diagnosis of allergic rhinitis was 17 years. One subject in the PL/CAL group had a diagnosis of atopic dermatitis 15 years prior to screening, and none of the subjects had a diagnosis of active asthma at screening. The mean for highest FEV_1 at screening was 99% (range: 82% to 131%) of predicted FEV_1 for the study population. Treatment sequences were comparable at screening, and results were similar for the FAS and safety population.

EFFICACY RESULTS:

For the primary efficacy endpoint and each secondary efficacy endpoint, CAL-101 was numerically superior to placebo for the combined treatment periods. Differences between treatments generally were statistically significant for the combined treatment periods; however, sequence and period effects were statistically significant in several analyses.

The mean (SD) change from baseline in the TNSS for the combined treatment periods was 5.8 (2.7) for CAL-101 and 7.4 (1.9) for placebo. The difference between treatments (95% CI) was -1.78 (-2.53, -1.03). The treatment effect was statistically significant ($p<0.001$), indicating that CAL-101 effectively inhibited nasal allergic rhinitis symptoms following an environmental chamber allergen challenge. The period effect was statistically significant ($p=0.002$), and the sequence effect approached statistical significance ($p=0.052$).

For subjects in the CAL/PL sequence, the mean (SE) change from baseline was 6.88 (0.46) for Period 1 (CAL) and 7.44 (0.46) for Period 2 (PL). The difference (95% CI) between treatments [-0.56 (-1.87; 0.75)] was not statistically significant ($p=0.395$). The mean (SE) change from baseline for subjects in the PL/CAL sequence was 7.43 (0.56) during Period 1 (PL) and 4.43 (0.56) during Period 2 (CAL). The difference (95% CI) between treatments [-3.00 (-4.60; -1.41)] for this sequence was statistically significant ($p=0.001$).

The following descriptions are for the combined treatment periods.

The mean (SD) change from baseline in TNNSS was 2.3 (2.2) for CAL-101 and 2.7 (2.4) for placebo. The difference (95% CI) between treatments was -0.48 (-1.14, 0.18). The treatment effect was not statistically significant ($p=0.146$). The period effect was statistically significant ($p=0.023$).

For the TSS, the mean (SD) change from baseline was 8.0 (4.4) for CAL-101 and 10.1 (3.5) for placebo. The difference (95% CI) between treatments was -2.26 (-3.35, -1.17). The treatment effect was statistically significant ($p<0.001$), as was the period effect ($p=0.001$).

The mean (SD) change from baseline in average nasal airflow (2-4 hours) was -93.4 (116.7) cm^3/sec for CAL-101 and -162.1 (141.1) for placebo. The difference (95% CI) between treatments was 72.27 (15.53, 129.0). The treatment effect was statistically significant ($p=0.014$). Neither the sequence effect ($p=0.534$) nor the period effect ($p=0.107$) was statistically significant. The mean (SD) change from baseline for 0-4 hours after dosing was -84.7 (111.7) cm^3/sec for CAL-101 and -150.4 (125.0) cm^3/sec for placebo.

For nasal secretion weight, the mean (SD) change from baseline was 14.7 (16.1) g for CAL-101 and 24.8 (15.7) g for placebo. The difference (95% CI) between treatments was -10.2 (-14.2, -6.24). The treatment effect was statistically significant ($p<0.001$). Neither the sequence effect ($P=0.087$) nor the period effect ($p=0.454$) was statistically significant.

The mean (SD) change from baseline in nasal congestion was 1.6 (0.7) for CAL-101 and 2.0 (0.6) for placebo. The difference (95% CI) between treatments was -0.42 (-0.65, -0.19). The treatment effect was statistically significant ($p=0.001$). The period effect was statistically significant ($p=0.002$).

The cytology of nasal scrapings before and after dosing and the change from baseline were comparable for CAL-101 and placebo.

PHARMACOKINETIC RESULTS:
On the first day CAL-101 was administered (Visit 2 for CAL/PL; Visit 4 for PL/CAL), the mean plasma concentration was 1087 ng/mL and 793 ng/mL at 1.5 hours and 3 hours after dosing, respectively. On the last day CAL-101 was administered (Visit 3 for CAL/PL; Visit 5 for PL/CAL), the mean plasma concentration prior to dosing and at the end of the allergen challenge (4-5 hours after dosing) was 474 ng/mL and 844 ng/mL, respectively.

PHARMACODYNAMIC RESULTS:
PD assessments were performed for 37 subjects following treatment with CAL-101 and for 19 subjects after treatment with placebo. The mean change from baseline in the relative percent of CD63+/CCR3+ cells 1.5 hours and 3 hours after the Day 1 morning dose was -76.3% and -65.5%, respectively, for CAL-101 and -9.9% and -5.7%, respectively, for placebo. These results indicate that CAL-101 inhibited ex vivo basophil activation.

SAFETY RESULTS:
No subjects died, experienced an SAE, or discontinued prematurely due to TEAEs. During treatment with CAL-101, 2/39 (5.1%) subjects had a total of 4 TEAEs, which included nasopharyngitis (2 events), oral herpes, and myalgia. All TEAEs were considered to be of mild intensity and not related to study medication.

During treatment with placebo, 4/41 (9.8%) subjects had a total of 14 TEAEs, which included fatigue, laryngitis, increased hepatic enzymes, headache, cough, nasal discomfort, and throat irritation. One subject had 4 occurrences of headache. All other TEAEs were single occurrences. All TEAEs were considered to be of mild intensity and not related to study medication.

No clinically relevant changes were observed in clinical laboratory tests, vital signs, FEV_1 or ECGs. For the combined treatment periods, the observed means were comparable between treatments, and the mean changes from baseline were small and comparable between treatments at each study visit. Within treatment sequences, the observed means and the mean changes from baseline were small and consistent between study periods.

CONCLUSION:
CAL-101 was significantly superior to placebo in reducing the allergic response following an environmental allergen challenge in a Vienna Challenge Chamber, with improvements noted in nasal symptoms, nasal airflow and nasal secretion weight. CAL-101 was well tolerated when administered orally at a dose of 100 mg twice daily over 7 days.

DATE OF THE REPORT: 03 August 2009