ARTICLE

REGN1908-1909 monoclonal antibodies block Fel d 1 in cat allergic subjects: Translational pharmacokinetics and pharmacodynamics

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Abstract

REGN1908-1909, a 1:1 cocktail of two fully human IgG_4 monoclonal antibodies (mAbs), REGN1908 and REGN1909, is being evaluated for treatment of cat allergy. Both REGN1908 and REGN1909 bind to the dominant cat allergen, Fel d 1. Adults with cat allergy confirmed by skin prick test (SPT) were randomized to single subcutaneous administration of placebo ($n = 6$) or REGN1908-1909 at doses of 150 ($n = 6$), 300 ($n = 6$), or 600 mg ($n = 6$). Blood samples were taken at prespecified time points for pharmacokinetic (PK) analysis and exploratory evaluation of biomarkers (IgE and SPT). Safety was assessed. Drug concentration-time profiles in serum for ascending doses of REGN1908-1909 were consistent with linear PKs. Noncompartmental analysis showed that maximum concentration (C_{max}) and exposure increased proportionately with dose, with similar time to maximum concentration (T_{max}) for REGN1908 and REGN1909 (6.2 to 8.2 days across doses), and a longer terminal half-life for REGN1908 (~ 30 days) relative to REGN1909 (~ 21 days). Adverse events were not dose dependent; there were no dose-limiting toxicities. REGN1908-1909 is characterized by linear and dose-proportional kinetics of the two individual mAb components. A single 600 mg dose maintains total mAb mean concentrations in serum above the target (mean of ~ 10 mg/L) for 8–12 weeks. Maintaining this mean target concentration resulted in translational pharmacodynamic effects: maximal mast cell degranulation in a passive cutaneous anaphylaxis mouse model, and maintenance of clinical efficacy measured by Total Nasal Symptom Score in a previous proof-of-mechanism study.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
REGN1908 and REGN1909 are fully human IgG_4 monoclonal antibodies that demonstrated high affinity, noncompetitive binding to distinct epitopes of Fel d 1 allergen. Whereas a cocktail of these monoclonal antibodies, REGN1908-1909, is being evaluated as a passive immunization strategy for treatment of cat allergy, its pharmacokinetics (PKs) have not been fully characterized, especially with respect to potential pharmacodynamic (PD) effects.

WHAT QUESTION DID THIS STUDY ADDRESS?
This first-in-human study of REGN1908-1909 evaluated the PKs and safety of REGN1908-1909 in cat-allergic individuals who were otherwise healthy. Translational context is provided by discussing the PK profile with regard to PD effects observed in mouse models and among patients in a proof-of-mechanism study.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
The PK profile of subcutaneous administration of REGN1908-1909 in subjects with cat allergy appeared to be dose-proportional, with half-lives of the individual components within the expected range for a typical monoclonal antibody. The concentration-time curve was consistent with previously reported peak PD effects, including clinical response that temporally coincides with the time of peak concentrations of the drugs in serum.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
Pharmacokinetic results support REGN1908-1909 600 mg as the dose that maintains drug concentrations in serum above a mean target concentration required for PD effects, and suggest a prophylactic dosing regimen of every 2–3 months.

INTRODUCTION

Cat allergy, one of the most common allergic disorders, has been estimated to be present in ~ 12% of the United States population greater than or equal to 6 years of age.[1] The dominant cat allergen is the secretoglobulin Fel d 1, which elicits IgE-mediated allergic responses in up to 95% of cat-allergic individuals,[2,3] resulting in symptoms of variable severity that include sneezing, runny nose, nasal itching, nasal congestion, conjunctivitis, and/or asthma. These symptoms may persist with continuous cat exposure and can be severe even with intermittent exposure.

Therapies for cat allergy are limited, with first-line treatment symptomatically driven and allergen-specific immunotherapy (AIT) the only disease-modifying strategy currently available. The proposed mechanism of AIT is induction of IgG antibodies that compete with IgE for allergen binding, thus inhibiting downstream mediators of inflammation.[4–7] However, AIT is generally restricted to individuals with severe and/or long duration allergy who do not respond to symptomatic treatment and have no contraindications.[8,9] Furthermore, AIT requires treatment of up to 5 years; is associated with side effects, including life-threatening events, such as asthma exacerbation and anaphylaxis; and demonstrates inconsistent efficacy.[10] The variable efficacy is

thought to result from heterogeneity of the induced IgG that may reflect immunoreactive rather than functional immunoglobulin.[11,12] Consequently, safer and more effective treatment options are needed.

REGN1908-1909 is a cocktail (1:1 ratio) of two fully human IgG_4 monoclonal antibodies (mAbs), REGN1908 and REGN1909, both of which demonstrated high affinity, noncompetitive binding to distinct epitopes of Fel d 1 allergen.[11,13] REGN1908-1909 is being evaluated as a passive immunization strategy for treatment of cat allergy. Although passive immunotherapy against antigens is an approach that has long been recognized,[14] the use of allergen-specific mAbs for this purpose is novel. REGN1908-1909 has previously been shown to increase the IgG/IgE ratio and reduce the allergic response in mice, and block Fel d 1 allergen binding to IgE.[11] The ability of REGN1908-1909 to block allergen binding with IgE prevents cross-linking of allergen-specific IgE:Fc-epsilon receptor complexes on mast cells and basophils; it is this cross-linking that results in effector cell degranulation and release of histamine and other inflammatory mediators that propagate the early allergic response. In a proof-of-mechanism study, a single dose of REGN1908-1909 reduced clinical symptoms in response to nasal provocation in cat-allergic subjects, with effects observed as early as day 8 after treatment that were maintained

until day 85.[11] The onset, magnitude, and duration of these clinical effects were suggested to coincide with antibody concentrations, and REGN1908-1909 was well-tolerated.

This analysis reports results from the first-in-human study that evaluated the pharmacokinetics (PKs) and safety of REGN1908-1909 in cat-allergic individuals who were otherwise healthy. Translational context is additionally provided by discussing the PK profile with regard to pharmacodynamic (PD) effects shown by the exposure-response relationship for inhibition of mast cell degranulation in a mouse passive cutaneous anaphylaxis (PCA) model, and by the clinical improvements among patients in the proof-of-mechanism study.[11,13]

METHODS

Study design

This was a randomized, placebo-controlled, double-blind, phase I study of the safety and tolerability of single ascending doses of REGN1908-1909 with evaluation of PK as a secondary objective (NCT01922661). This study was approved by the sponsor (Regeneron Pharmaceuticals) and Ethics Committees (Stichting Beoordeling Ethiek Biomedisch Onderzoek, The Hague, The Netherlands; Health and Disability Ethics Committees, Wellington, New Zealand; Regional Ethical Review Board in Lund, Lund, Sweden; Health Research Authority – National Research Ethics Service, Manchester, United Kingdom), and was conducted in accordance with the Declaration of Helsinki; all subjects provided written informed consent prior to participation. The safety oversight team was coordinated by the study sponsor (Regeneron) and met periodically to review blinded safety data.

The study consisted of a screening period to determine study eligibility; a 4-day in-clinic visit during which study treatment was administered with follow-up to monitor acute safety and tolerability; and follow-up outpatient visits and laboratory evaluations on days 8, 15, 22, 29, 43, 57, 85, and 113.

Study population

Subjects were adults between 18 and 55 years of age, inclusive, who self-reported a history of cat allergy but were otherwise healthy. Allergy to cats was confirmed during the screening period by a positive skin prick test (SPT) using standardized cat hair extract (wheal diameter ≥ 5 mm at $\leq 10,000$ allergy units [AUs]), a positive Fel d 1-specific IgE test (≥ 0.35 kAU/L), and a negative galactose-alpha-1, 3-galactose-specific IgE antibody test (≤ 0.35 kAU/L), a common cause of false-positive tests to standardized cat hair extract.[15] Additional inclusion criteria were a body mass index between 18.0 kg/m^2 and 32.0 kg/m^2, inclusive, and willingness to practice adequate contraception among sexually active men and women of childbearing potential.

Key exclusion criteria included a persistent, chronic, or active recurring infection requiring treatment with antibiotics, antivirals, or antifungals within 4 weeks prior to screening; enzyme or laboratory findings indicative of organ dysfunction or any clinically significant deviation from the normal range; use of any concomitant medications 7 days or at least 5 half-lives (whichever was longer) prior to screening; history of asthma; and having received live/attenuated vaccinations within 12 weeks prior to screening. A positive pregnancy test or the presence of a medical or psychiatric condition that would place the subject at risk and/or would interfere with study participation or interpretation of results were also reasons for exclusion.

Cohorts and dosing

Subjects were randomized 3:1 REGN1908-1909:placebo into three sequential, ascending, single subcutaneous (s.c.) dose cohorts of REGN1908-1909 150 mg, 300 mg, and 600 mg. Lyophilized REGN1908 and REGN1909 were supplied separately, and reconstituted with 2.3 ml of sterile water for a final concentration of 100 mg/ml REGN1908 or 100 mg/ml REGN1909 in histidine, polysorbate 80, and sucrose (pH 5.8). The reconstituted mAbs were mixed 1:1 resulting in a concentration of 100 mg/ml total REGN1908-1909; the 150 mg dose was administered as a single injection, with the 300 mg and 600 mg doses administered as two and three injections, respectively. Placebo was provided in matching vials and reconstituted with sterile water. All injections were s.c. in the abdominal region using different quadrants when multiple injections were required.

All subjects in a cohort were observed for at least 15 days for dose-limiting toxicities (DLTs) before the next increased dose was initiated. A DLT was defined as a treatment-related toxicity grade greater than or equal to 3 based on US Food and Drug Administration (FDA) guidance,[16] or any physical or laboratory finding that triggered diagnostic evaluation and therapeutic intervention by a medical subspecialist.

Pharmacokinetic evaluation

Blood was collected during the in-clinic period on day 1 (predose) and on days 2 and 4 (24 ± 1 and 72 ± 2 h postinjection, respectively), and at each subsequent visit, including end of study (day 113) or early termination.

Serum samples were analyzed by the sponsor for total REGN1908 and total REGN1909 using two independent

validated enzyme-linked immunosorbent assays (ELISA) that detect these mAbs regardless of target occupancy. The assays use microtiter plates coated with a mouse anti-REGN1908 or anti-REGN1909 antibody as capture reagents, with REGN1908 or REGN1909 as standards, respectively. The standards, quality controls (QCs), and samples were added to the plate and the captured drug on the plate was detected by a biotinylated mouse anti-human IgG_4 mAb, followed by NeutrAvidin conjugated to horseradish peroxidase (HRP; NeutrAvidin-HRP). A luminol-based substrate specific for peroxidase is then added to achieve a signal intensity that is proportional to the concentration of total REGN1908 or REGN1909. The relative light units (RLUs) of the standards are calibrated against their respective nominal concentrations using a weighted four-parameter logistic regression model from which all other readings (samples and QCs) are computed. The lower limit of quantification (LLOQ) for both REGN1908 and REGN1909 assays in human serum is 0.078 mg/L.

Both assays were validated and the assay performance was evaluated based on several standard validation parameters, including inter- and intra-assay accuracy, precision, and linearity. Assay robustness, defined as a measure of the capacity of the analytical method to remain unaffected by deliberate variations in method conditions, and assay ruggedness, defined as the degree of reproducibility of test results obtained by analysis of samples under a variety of test conditions, were demonstrated. All parameters were validated in accordance with and within the specifications as recommended in the Guidance for Industry on Bioanalytical Method Validation by the FDA.[17]

Exploratory biomarker analysis

The SPT was conducted with increasing concentrations (30–10,000 bioequivalent AU/ml) of cat hair extract at screening and days 8, 29, and 113 or early termination, and the wheal-and-flare responses (average of the longest and the longest orthogonal diameter) was measured 15 min after administration; the mean diameter of duplicate tests was calculated. Histamine was used as a positive control and diluent was used as a negative control without duplication to minimize the number of skin pricks.

Serum samples were collected predose (day 1) and on days 8, 29, and 113 for determination of total IgE and IgE-specific for cat dander, Fel d 1, Fel d 2, and galactose-1, 3-alpha-galactose. Analyses were conducted at a central laboratory (Viracor-IBT Laboratories, Lee's Summit, MO, USA) using ImmunoCAP assay (ThermoFisher Scientific, Waltham, MA, USA).

Safety

Evaluation of safety and tolerability was based on reporting of treatment-emergent adverse events (TEAEs) regardless of

causality, with additional assessment to determine whether the events were treatment-related. Evaluations also included physical examination, clinical laboratory tests, vital signs, and electrocardiography. Assessment included evaluation for maximum tolerated dose (MTD), defined as the dose immediately below the level at which dosing was stopped due to the occurrence of one or more DLTs.

Statistical analysis

As there was no formal statistical hypothesis, power calculations were not conducted; sample size was consistent with a phase I safety study. The PK population consisted of all patients who received REGN1908-1909 or placebo and who had at least one study drug measurement subsequent to administration, and the safety population consisted of all randomized patients who received any study drug or placebo.

For the PK analysis, noncompartmental parameters were calculated using Phoenix WinNonlin (version 6.2; Pharsight Inc.) and the model 200 (extravascular injection) based on the linear trapezoidal rule and linear interpolation with uniform weighting. Concentrations of REGN1908 and REGN1909 in serum below the LLOQ were set to zero. Descriptive statistics for PK parameters are presented by dose group.

For the SPT, wheal diameter was compared versus placebo at each evaluated time point for all dose levels combined and by individual doses.

RESULTS

Population and cohorts

Of the 117 subjects who were screened, 24 were enrolled, randomized (18 REGN1908-1909, 6 placebo), and treated, and these subjects were included in both the safety and PK populations. Subjects were predominantly men (70.8%) and Black/African Americans (70.8%), with a mean age of 31.1 years, and the demographic characteristics were generally similar across the treatment groups (Table 1). Twenty subjects completed the study; reasons for withdrawal were lost to follow-up ($n = 3$) and noncompliance with protocol ($n = 1$).

Pharmacokinetics

Drug concentration-time profiles in serum after a single s.c. dose of REGN1908-1909 were characterized by linear and dose proportional kinetics for both REGN1908 and REGN1909 (Figure 1). The initial absorption phase, with a maximum concentration (C_{max}) at ~ 7 days, was followed by a mono-exponential elimination phase, with concentrations at

all time points, including the final evaluation at nominal day 112, exceeding the LLOQ (mean concentrations for each study day are presented in Table S1). Summary statistics (Table 2) showed that time to C_{max} (T_{max}) was similar for REGN1908 and REGN1909, and ranged from 6.2 to 8.2 days across doses. The dose-proportionality described by the concentration-time profiles (area under the concentration-time curve from zero to infinity [AUC_∞]) was paralleled by the estimated C_{max} mean (coefficient of variation percentage [CV%]) concentrations at the 150, 300, and 600 mg doses of REGN1908-1909 of 8.1 mg/L (21.5%), 17.1 mg/L (22.4%), and 30.2 mg/L (50.6%), respectively, for REGN1908, and 11.0 (16.5%), 18.0 (19.7%), and 42.5 (52.8%), respectively, for REGN1909 (Table 2). These C_{max} values resulted in total concentrations of the combined mAbs of 19.1, 35.1, and 72.7 mg/L at the three doses of the REGN1908-1909 cocktail, respectively. Dose proportionality

TABLE 1 Demographic characteristics of the study population

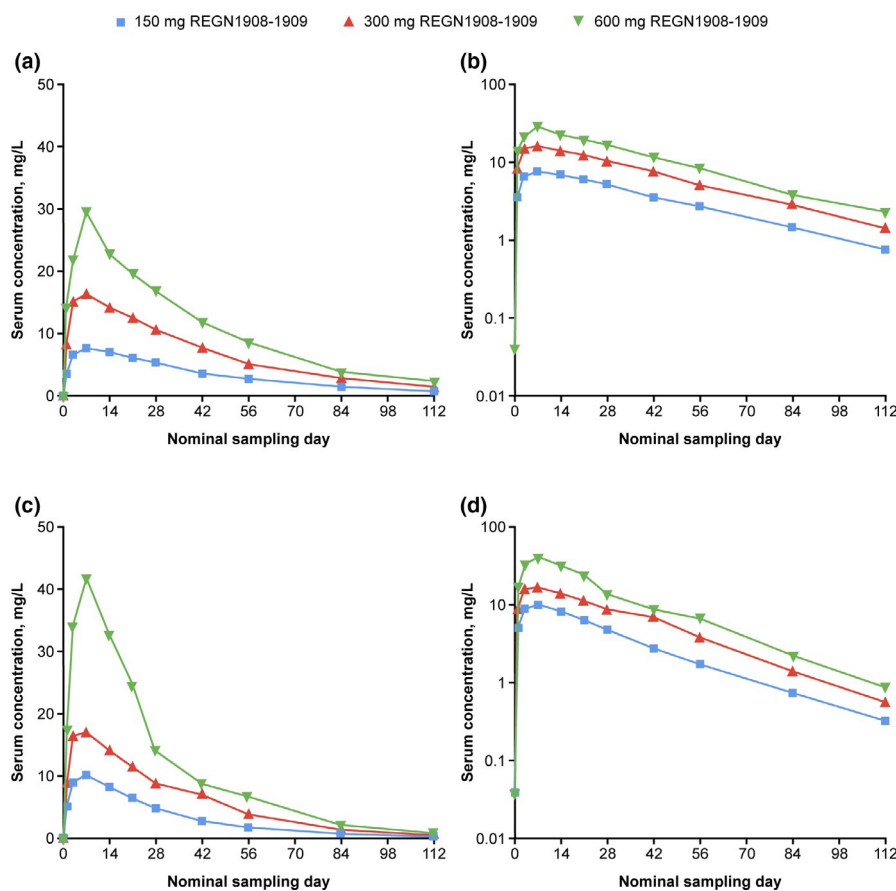| | | | REGN1908-1909 | | | |
Variable	Total (N=24)	Placebo (n=6)	150 mg (n=6)	300 mg (n=6)	600 mg (n=6)	All doses (n=18)
Age, years, mean (SD)	31.1 (9.7)	31.3 (10.3)	28.7 (8.4)	27.8 (6.0)	36.7 (12.9)	31.1 (9.9)
Males, n (%)	17 (70.8)	3 (50.0)	4 (66.7)	4 (66.7)	6 (100)	14 (77.8)
Race, n (%)						
Asian	1 (4.2)	1 (16.7)	0	0	0	0
Black/African-American	17 (70.8)	5 (83.3)	3 (50.0)	6 (100)	3 (50.0)	12 (66.7)
White	6 (25.0)	0	3 (50.0)	0	3 (50.0)	6 (33.3)
BMI, kg/m^2, mean (SD)	25.5 (3.8)	25.3 (2.9)	23.7 (4.1)	25.9 (4.3)	27.0 (3.9)	25.5 (4.1)

Abbreviation: BMI, body mass index.

FIGURE 1 Concentration-time curves of the monoclonal antibodies REGN1908 and REGN1909 in serum after a single subcutaneous dose of REGN1908-1909 (1:1 cocktail of the two monoclonal antibodies). (a) REGN1908 linear scale. (b) REGN1908 log scale. (c) REGN1909 linear scale. (d) REGN1909 log scale. Concentrations below the lower limit of quantification (LLOQ; 0.0780 mg/L) are imputed as LLOQ/2 = 0.0390 mg/L

TABLE 2 Summary statistics of pharmacokinetic parameters after single subcutaneous dose of REGN1908-1909

	Mean (CV%)					
	REGN1908-1909 150 mg (75 mg REGN1908 + 75 mg REGN1909)		REGN1908-1909 300 mg (150 mg REGN1908 + 150 mg REGN1909)		REGN1908-1909 600 mg (300 mg REGN1908 + 300 mg REGN1909)	
Parameter	REGN1908	REGN1909	REGN1908	REGN1909	REGN1908	REGN1909
C_{max}, mg/L	8.1 (21.5)	11.0 (16.5)	17.1 (22.4)	18.0 (19.7)	30.2 (50.6)	42.5 (52.8)
C_{max}/Dose,[a] 1/L	0.108 (21.5)	0.147 (16.5)	0.114 (22.4)	0.120 (19.7)	0.101 (50.6)	0.142 (52.8)
C_{last}, mg/L	0.8 (37.7)	0.3 (54.3)	1.6 (48.2)	0.7 (59.6)	2.4 (34.4)	0.9 (37.7)
T_{max}, days	6.9 (59.3)	6.2 (69.7)	7.6 (47.7)	6.2 (70.4)	8.2 (34.8)	8.2 (34.8)
T_{last}, days	111 (1.2)	107 (10.5)	107 (10.6)	107 (10.6)	111 (1.1)	111 (1.1)
AUC_{∞}, day•mg/L	408 (18.3)	359 (26.1)	836 (22.5)	697 (26.6)	1321 (30.3)	1278 (43.5)
AUC_{∞}/dose,[a] day/L	5.4 (18.3)	4.8 (26.1)	5.6 (22.5)	4.6 (26.6)	4.4 (30.3)	4.3 (43.5)
$t_{1/2}$, day	29.4 (16.1)	22.0 (21.5)	31.2 (26.2)	20.0 (12.8)	30.9 (26.3)	20.1 (14.4)
CL/F, L/day	0.189 (17.4)	0.224 (31.2)	0.186 (19.3)	0.228 (26.0)	0.247 (32.5)	0.274 (41.4)
Vss/F, L	9.3 (23.4)	6.5 (13.7)	8.4 (33.8)	6.6 (38.1)	9.3 (40.3)	8.1 (48.0)
MRT_{last}, day	35.5 (10.9)	26.5 (23.9)	31.2 (24.3)	26.4 (25.1)	34.7 (14.1)	26.6 (15.4)
MRT_{∞}, day	45.1 (16.8)	30.0 (26.7)	41.9 (29.4)	29.4 (26.1)	45.5 (24.5)	29.1 (17.9)

Abbreviations: AUC_{∞}, area under the curve from the time of dosing extrapolated to infinity; C_{last}, last quantifiable concentration; C_{max}, maximum concentration; CL/F, clearance; CV%, percentage coefficient of variation; MRT_{last}, mean residence time up to and including the last positive concentration; MRT_{∞}, mean residence time extrapolated to infinity; T_{last}, time of last quantifiable concentration; T_{max}, time to maximum concentration; $t_{1/2}$, terminal half-life; Vss/F, volume of distribution at steady-state.

[a]Dose-normalized.

TABLE 3 Treatment-emergent adverse events

	Number (%) of subjects					
			REGN1908-1909			
TEAE	Total (N = 24)	Placebo (n = 6)	150 mg (n = 6)	300 mg (n = 6)	600 mg (n = 6)	All doses (n = 18)
At least 1 TEAE	14 (58.3)	4 (66.7)	4 (66.7)	2 (33.3)	4 (66.7)	10 (55.6)
Serious TEAEs	0	0	0	0	0	0
Discontinuations due to TEAE	0	0	0	0	0	0
Most common TEAEs (≥2 subjects in total population)						
Upper respiratory tract infection	3 (12.5)	1 (16.7)	1 (16.7)	0	1 (16.7)	2 (11.1)
Myalgia	2 (8.3)	0	2 (33.3)	0	0	2 (11.1)
Contusion	2 (8.3)	0	1 (16.7)	0	1 (16.7)	2 (11.1)
Ligament sprain	2 (8.3)	0	1 (16.7)	1 (16.7)	0	2 (11.1)
Treatment-related TEAEs	6 (25.0)	1 (16.7)	1 (16.7)	1 (16.7)	3 (50.0)	5 (27.8)

Abbreviation: TEAE, treatment-emergent adverse event.

was further supported by the estimated dose normalized C_{max} and AUC_{∞} values (Table 2).

Apparent clearance (CL/F) was slightly lower for REGN1908 than REGN1909, and conversely, the (terminal half-life [$t_{1/2}$]) was lower for REGN1909 relative to REGN1908 (Table 2), with consistent values across dose groups for each mAb; overall $t_{1/2}$ was determined to be $\sim 30 \pm 7$ days and 21 ± 3 days for REGN1908 and REGN1909, respectively.

Similarly, lower mean residence times were observed for REGN1909 relative to REGN1908 (Table 2).

Biomarkers

Concentrations of total IgE remained stable in all cohorts across evaluated time points, although there was wide

variability (large SDs) and the 150 mg cohort had higher mean concentrations than the other cohorts (Figure S1).

On the SPT, placebo-treated subjects generally showed a consistent response at all time points, whereas wheal diameter among subjects treated with REGN1908-1909 was lower than baseline diameter (Figure S2a), and this trend was especially noted at higher concentrations of cat allergen (i.e., as cat hair extract increased), the average wheal diameter was lower with REGN1908-1909 treatment (Figure S2b). However, differences were not significant versus placebo, and no dose response was observed; the lowest dose appeared to have the highest response.

Safety

A total of 14 subjects (58.3%) had TEAEs, and none of the subjects discontinued due to a TEAE (Table 3). No DLTs were observed and the MTD was not reached; none of the TEAEs was serious and all were of mild or moderate severity. The most common TEAEs overall (≥2 subjects of total population) included upper respiratory tract infection (12.5%), myalgia (8.3%), contusion (8.3%), and ligament sprain (8.3%; Table 3); except for an upper respiratory tract infection in a placebo-treated subject, these TEAES occurred in the REGN1908-1909 treated groups but were not dose-dependent. TEAEs that were deemed related to treatment were reported in six subjects (1 placebo and 5 REGN1908-1909), with injection site reactions the most frequent treatment-related TEAEs ($n = 3$; all with REGN1908-1909). Other treatment-related TEAEs were myalgia, nasal congestion, somnolence, and oral hypoesthesia (placebo subject). All treatment-related TEAEs resolved without medication. There were no clinically meaningful trends in any of the other safety outcomes including physical examination, clinical laboratory tests, vital signs, and electrocardiography.

DISCUSSION

REGN1908-1909 represents a potentially new approach for reduction of cat allergy symptoms by directly targeting the major cat allergen, Fel d 1. This first-in-human study characterized the PK of REGN1908-1909 in subjects with cat allergy and provides support for the favorable safety profile previously described.[11]

Single s.c. injections of REGN1908-1909 at the three dose levels resulted in C_{max} and total exposure (AUC) values that indicated linear and dose-proportional PK of both mAb components with increasing dose of the parent cocktail. The T_{max} of 8.2 days and the concentration in serum of 72.7 mg/L for the total of the two antibodies at the 600 mg dose were also consistent with what has been reported in the proof-of-mechanism study, 8 days and

74.4 mg/L,[13] respectively, demonstrating robustness with regard to both the assay methodology and the PK of REGN1908-1909 at this dose level. Indeed, the concentration-time profile of the 600 mg dose in the current analysis appeared comparable with that previously reported in the proof-of-mechanism study,[13] showing bi-exponential PK and concentrations that were similar across time points. Although the concentration in serum was evaluated to nominal day 84 in the proof-of-mechanism study, the last time point assessed in the current analysis was nominal day 112, and concentrations of the individual mAbs (2.4 and 0.9 mg/L for REGN1908 and REGN1909, respectively) still substantially exceeded the LLOQ (0.078 mg/L).

To further probe for potential differences in the REGN1908 and REGN1909 PK profiles between the two studies, a post hoc comparison was conducted of PK parameters using an unpaired t-test (GraphPad Prism version 8.4.1). Although no differences were observed between the trials for REGN1909, statistical differences were noted for REGN1908 for dose-normalized C_{max} and AUC_{∞}, and for CL/F and volume of distribution at steady-state (Vss/F; Table S2), However, the interplay in differences in CL/F and Vss/F results in a half-life for REGN1908 that was comparable in both studies, and would lend to similar concentrations of REGN1908. Because in vitro and proof-of-mechanism studies have shown that both mAbs are required to achieve maximal effects,[11,13] these differences are likely to be clinically inconsequential for overall efficacy.

The half-life of REGN1908 is slightly longer than that of REGN1909, and both half-lives are within the range expected for a typical mAb for an exogenous target.[18] Small differences in other PK parameters were also observed between REGN1908 and REGN1909. These differences, especially with regard to the half-lives of the mAbs, are consistent with REGN1908 having a higher affinity than REGN1909 for natural Fel d 1.[11] However, these differences are unlikely to be of clinical relevance, as the proof-of-mechanism study showed that the presence of both mAbs results in greater activity than each mAb alone, suggesting a synergistic relationship that relies on binding multiple Fel d 1 epitopes to prevent Fel d 1-induced crosslinking of polyclonal IgE.[11,13]

Results from the exploratory biomarker analysis showed that REGN1908-1909 did not affect IgE levels, which was not unexpected given that this was a single dose study with a relatively short follow-up period. Although it is possible that longer exposure may potentially downregulate endogenous IgE production, this would need to be evaluated in multiple dose studies. Nevertheless, the correlation observed between concentrations of two IgE biomarkers in serum at baseline, cat dander-specific IgE and anti-Fel d 1 IgE, validated the subject selection criteria, because the cat dander test is standardized by Fel d 1 content.

REGN1908-1909 administration resulted in a slight reduction in SPT responses, especially at higher allergen concentrations. However, no dose response was observed, likely
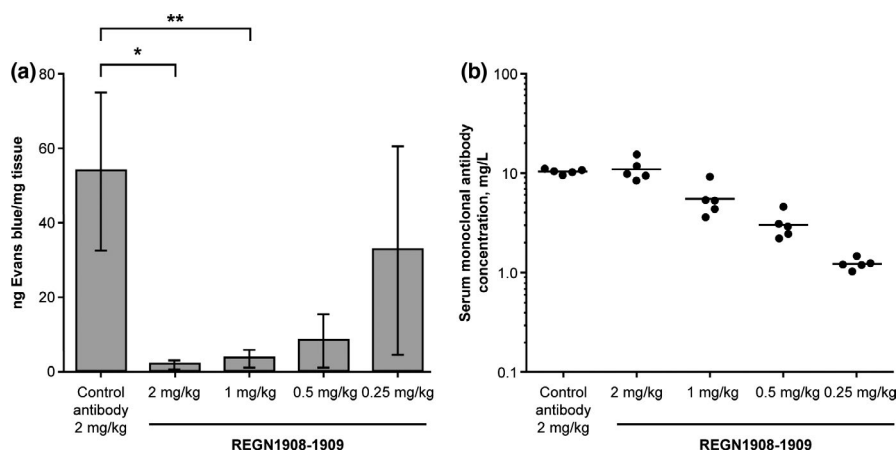
FIGURE 2 Monoclonal antibody concentrations in serum and their relationship to inhibition of mast cell degranulation inhibition induced by cat hair extract in a passive cutaneous anaphylaxis mouse model of IgE-mediated early allergic response (immediate hypersensitivity). Mast cell degranulation is proportional to the amount of Evans blue recovered from the tissue. Panel a adapted with permission from Orengo et al.[11] (licensed under CC BY 4.0.) ***$p < 0.001$ and *$p < 0.05$ versus placebo

because of the large variability in wheal size and the small sample size that also precluded more formal evaluation of PD effects. To provide PD context in the absence of such an evaluation in the current study, a translational approach can be used by relating the observed PK profile to PK and PD data from other published studies, including a mouse model and the proof-of-mechanism study. The proof-of-mechanism study, in particular, evaluated PK of a single 600 mg s.c. dose concurrently with clinical signs and symptoms in cat-allergic subjects who were treated with REGN1908-1909 who were then subsequently evaluated using nasal antigen challenge with cat hair extract.[11,13] As already stated, the PK in the current analysis was consistent with what has been reported in the proof-of-mechanism study,[13] further suggesting the appropriateness of extrapolating the PK findings from the current study to PD effects. The key translational assumption, as discussed below, is that the mean concentration associated with maximal inhibition of mast cell degranulation, a downstream marker of type 1 hypersensitivity reactions, in the mouse PCA model is also the mean target concentration above which maximal clinical response is induced and maintained after nasal allergen challenge in subjects with cat allergy.

Using this translational approach, the concentration-time curve of the REGN1908-1909 600 mg dose in the proof-of-mechanism study was observed to be in temporal concordance with the clinical response across efficacy end points after nasal antigen challenge.[11,13] These end points included the objective measure of peak nasal inspiratory flow as well as the patient-reported outcomes of Total Nasal Symptom Score (nasal congestion, sneezing, nasal itching, and runny nose) and a nasal symptoms visual analog scale. The magnitude of improvement on all three measures was concurrent with the PK profile, with peak improvements in nasal

symptom that coincided with peak concentrations, and durability of response of ~ 3 months (85 days).

The above observations support a direct relationship between PK and PD, which show that there is no delay in clinical effects upon drug target engagement with Fel d 1 allergens, and provide evidence that the PK profile of REGN1908-1909 is unaffected by antigen challenge. Importantly, at the 600 mg dose of REGN1908-1909, total concentrations of both mAbs combined in serum remained above greater than 10 mg/L mean concentration throughout the proof-of-mechanism study including at day 84,[13] and was greater than 10 mg/L until day 84 (6.1 mg/L) in the current study. This mean target concentration is especially relevant because it is consistent with the mean concentration that has previously been reported to result in significant and substantial inhibition of mast cell degranulation in response to challenge with cat hair extract in a PCA mouse model of IgE-mediated early allergic response (immediate hypersensitivity; Figure 2)[11]; mast cell degranulation is a key component of the acute allergic response and may play a role in chronic allergy.[19] Although a mean drug concentration of 10 mg/L (2 mg/kg dose) in serum resulted in maximum inhibition of mast cell degranulation in the PCA model, even a mean concentration of ~ 6 mg/L (1 mg/kg dose) was associated with almost complete inhibition (Figure 2). Therefore, it can be proposed that if time above a mean concentration (e.g., 10 mg/L) is the driver of protection to allergen, the 600 mg dose would be expected to provide protective coverage over a period of ~ 8–12 weeks, thereby also suggesting a potential dosing interval. Both the $t_{1/2}$ and mean residence time additionally support the duration of response and the proposed dosing regimen.[11,13]

REGN1908–1909 was well-tolerated, and the incidence of TEAEs was not dose-dependent; no serious TEAEs occurred and none of the subjects discontinued due to a TEAE. Importantly, there were no DLTs and the MTD was not

reached. Of the few treatment-related TEAEs, half were injection site reactions.

Limitations of this study include the small sample size and the fact that the population may not necessarily be representative, because Black/African Americans may be overrepresented. Another limitation is that, because clinical end points were not included and the study was not statistically powered to enable PD analysis based on biomarkers, such analysis relied on data from another study. However, as discussed above, the PK profile in the current analysis is consistent with that study, indicating robustness and supporting the translational approach to characterizing the relationship between the PK and PD effects of REGN1908-1909.

CONCLUSIONS

The PK profile of REGN1908-1909, a 1:1 cocktail of REGN1908 and REGN1909, was characterized by linear and dose-proportional kinetics of the two individual mAb components. This profile was also concordant with a previous study and paralleled the reported clinical response, demonstrating a PK/PD relationship[13] and providing evidence that the 600 mg dose of REGN1908-1909 (300 mg REGN1908 + 300 mg REGN1909) is appropriate for maintaining the drug concentration in serum above a mean 10 mg/L target that results in PD effects. The elimination half-life and MRT additionally support the extended duration of observed clinical response, and combined with the drug-concentration profile, suggest that a dosing regimen of 2–3 months would be appropriate for the treatment of individuals with cat allergy. REGN1908-1909 was well-tolerated, consistent with what has previously been reported.[11] Further studies are warranted to evaluate the PKs, long-term safety, and efficacy associated with a multiple dose regimen of REGN1908-1909. Furthermore, the available data on REGN1908-1909 suggest that development of appropriate mAbs may enable passive immunization for the management of other common allergies, such as to birch or grass, by directly targeting their major antigens.

CONFLICTS OF INTEREST
All authors are employees and stockholders of Regeneron Pharmaceuticals, the sponsor of the study.

AUTHOR CONTRIBUTIONS
M.A.K., J.M.O., A.R., and J.D.D. wrote the manuscript. M.A.K., J.M.O., A.R., and J.D.D. designed the research. All authors performed the research and analyzed the data.

REFERENCES

1. Salo PM, Arbes SJ, Renee J et al. Prevalence of allergic sensitization in the United States: results from the National Health and Nutrition Examination Survey (NHANES) 2005–2006. *J Allergy Clin Immunol*. 2014;134:350-359.
2. van Ree R, van Leeuwen WA, Bulder I, Bond J, Aalberse RC. Purified natural and recombinant Fel d 1 and cat albumin in in vitro diagnostics for cat allergy. *J Allergy Clin Immunol*. 1999;104:1223-1230.
3. Gronlund H, Saarne T, Gafvelin G, van Hage M. The major cat allergen, Fel d 1, in diagnosis and therapy. *Int Arch Allergy Immunol*. 2010;151:265-274.
4. Wachholz PA, Durham SR. Mechanisms of immunotherapy: IgG revisited. *Curr Opin Allergy Clin Immunol*. 2004;4:313-318.
5. Jutel M, Jaeger L, Suck R, et al. Allergen-specific immunotherapy with recombinant grass pollen allergens. *J Allergy Clin Immunol*. 2005;116:608-613.
6. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *World Allergy Organ J*. 2015;8:17.
7. Shamji MH, Durham SR. Mechanisms of allergen immunotherapy for inhaled allergens and predictive biomarkers. *J Allergy Clin Immunol*. 2017;140:1485-1498.
8. Zuberbier T, Bachert C, Bousquet PJ, et al. GA(2) LEN/EAACI pocket guide for allergen-specific immunotherapy for allergic rhinitis and asthma. *Allergy*. 2010;65:1525-1530.
9. Bousquet J, Pfaar O, Togias A, et al. 2019 ARIA Care pathways for allergen immunotherapy. *Allergy*. 2019;74:2087-2102.
10. Dhami S, Agarwal A. Does evidence support the use of cat allergen immunotherapy? *Curr Opin Allergy Clin Immunol*. 2018;18:350-355.
11. Orengo JM, Radin AR, Kamat V et al. Treating cat allergy with monoclonal IgG antibodies that bind allergen and prevent IgE engagement. *Nat Commun*. 2018;9:1421.
12. Shamji MH, Ljørring C, Francis JN, et al. Functional rather than immunoreactive levels of IgG4 correlate closely with clinical response to grass pollen immunotherapy. *Allergy*. 2012;67:217-226.
13. Shamji MH, Singh I, Layhadi JA et al. Passive immunotherapy with single-dose of anti-Fel d 1 monoclonal antibodies REGN1908-1909 in cat allergen-induced allergic rhinitis. *Am J Respir Crit Care Med*. 2021;204(1):23-33.
14. Cooke RA, Barnard JH, Hebald S, Stull A. Serological evidence of immunity with coexisting sensitization in a type of human allergy (Hay Fever). *J Exp Med*. 1935;62:733-750.
15. Gronlund H, Adedoyin J, Commins SP, Platts-Mills TA, van Hage M. The carbohydrate galactose-alpha-1,3-galactose is a major IgE-binding epitope on cat IgA. *J Allergy Clin Immunol*. 2009;123:1189-1191.
16. U.S. Food and Drug Administration & Center for Biologics Evaluation and Research. Guidance for Industry. Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. 2007. https://www.fda.gov/media/73679/download. Accessed April 14, 2020.
17. U.S. Food and Drug Administration, Center for Biologics Evaluation and Research & Center for Veterinary Medicine (CVM). Bioanalytical Method Validation. Guidance for Industry. 2018. https://www.fda.gov/files/drugs/published/Bioanalytical-Method-Validation-Guidance-for-Industry.pdf. Accessed April 19, 2020.
18. Dong JQ, Salinger DH, Endres CJ, et al. Quantitative prediction of human pharmacokinetics for monoclonal antibodies: retrospective

analysis of monkey as a single species for first-in-human prediction. *Clin Pharmacokinet*. 2011;50:131-142.

19. Galli SJ, Tsai M. IgE and mast cells in allergic disease. *Nat Med*. 2012;18:693-704.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.