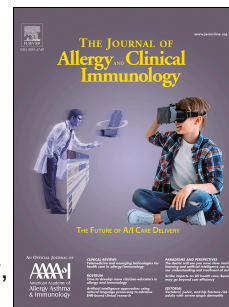


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Novel antibody cocktail targeting Bet v 1 rapidly and sustainably treats birch allergy symptoms in a Phase 1 study

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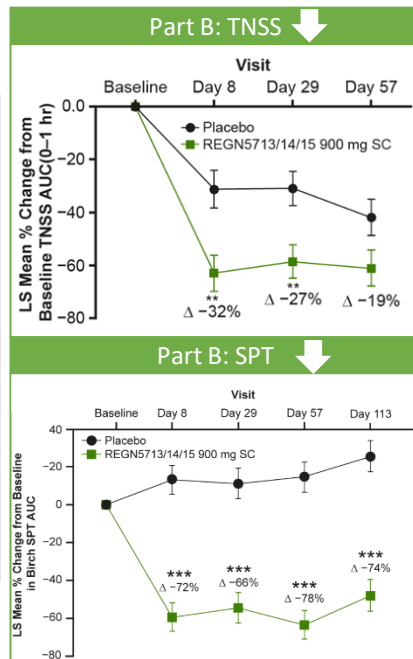
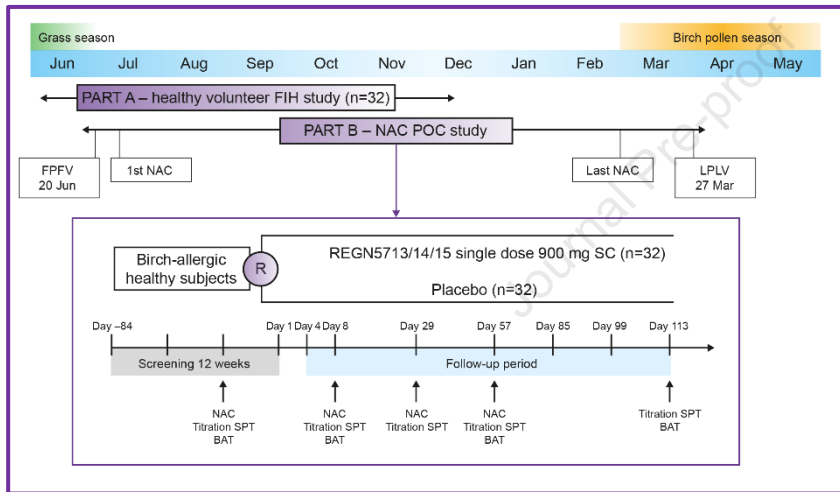
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Novel antibody cocktail targeting Bet v 1 rapidly and sustainably treats birch allergy symptoms in a Phase 1 study



AUC, area under the curve; BAT, basophil activation test; Bet v 1, major birch tree allergen; FIH, first in human; FPFV, first patient first visit; LS, least squares; NAC, nasal allergen challenge; POC, proof of concept; SC, subcutaneous; SPT, skin prick test; TNSS, total nasal symptom score.

Novel antibody cocktail targeting Bet v 1 rapidly and sustainably treats birch allergy symptoms in a Phase 1 study

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Gevaert phase 1 1857 study of REGN5713-5714-5715 in birch allergy JACI

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Abstract (250/250)

Background: The efficacy of an allergen-specific IgG cocktail to treat cat allergy suggests that allergen-specific IgG may be a major protective mechanism elicited by allergen immunotherapy.

Objective: Extending these findings, we tested a Bet v 1-specific antibody cocktail in birch-allergic subjects.

Methods: Phase 1, randomized, double-blind, study: Part-A, ascending doses of Bet v 1-specific antibody cocktail "REGN5713/14/15" (150–900 mg) in 32 healthy adults; Part-B, single subcutaneous 900 mg dose or placebo in 64 birch-allergic subjects. Total nasal symptom score (TNSS) response to titrated birch extract nasal allergen challenge (NAC) and skin prick test (SPT) with birch and alder allergen were assessed at screening and days 8, 29, 57 and 113 (SPT only); basophil activation tests (n=26) were conducted.

Results: Single dose REGN5713/14/15 significantly reduced TNSS following birch NAC relative to baseline. Differences in TNSS AUC(0–1 hr) versus placebo (day 8: -1.17 , $P = .001$; day 29: -1.18 , $P = .001$; day 57: -0.85 , $P = .024$) and titration SPT with birch difference in AUC of mean wheal diameters versus placebo (all $P < .001$) were sustained for ≥ 2 months; similar results observed with alder SPT. REGN5713/14/15 was well-tolerated. Basophil responsiveness to birch-related allergens was significantly decreased in REGN5713/14/15-treated subjects versus placebo on days 8, 57, and 113 (all $P < .01$).

Conclusion: Single dose REGN5713/14/15 was well tolerated and provided a rapid (1 week) and durable (2 months) reduction in allergic symptoms after birch allergen NAC, potentially offering a new paradigm for the treatment of birch allergy symptoms.

Clinical Trials Registration: Clinicaltrials.gov, NCT03969849. Registered May 31, 2019, <https://clinicaltrials.gov/ct2/show/NCT03969849>

Clinical Implications (words limit: 33/30)

A single dose of a Bet v 1-specific antibody cocktail, REGN5713/14/15, was well-tolerated and provided rapid (1 week), sustained (2 months) inhibition of birch and related allergy symptoms in a nasal provocation model.

Capsule Summary (word limit 37/35)

A single subcutaneous dose of a novel Bet v 1-specific antibody cocktail, REGN5713/14/15, may offer a rapid, sustained (2 months) and well-tolerated treatment of birch and related allergy symptoms based on Phase-1 results.

Key words: allergen immunotherapy; monoclonal antibodies; biologics; allergic rhinitis; Bet v 1; birch allergy.

Abbreviations used

AIT-	Allergen immunotherapy
AR-	Allergic rhinitis
AUC-	Area under the curve
BAT-	Basophil activation test
FEV1-	Forced expiratory volume in one second
IgE-	Immunoglobulin E
IV-	Intravenous
mAbs-	Monoclonal antibodies
MMRM-	mixed-effect model repeated measures
NAC-	Nasal allergen challenge
OAS-	Oral allergy syndrome
PD-	Pharmacodynamic
PK-	Pharmacokinetic
PNIF-	Peak nasal inspiratory flow
POM-	Proof of mechanism
SC-	Subcutaneous
SCIT-	Subcutaneous immunotherapy
SLIT-	Sublingual immunotherapy
SPT-	Skin prick test
TEAE-	Treatment-emergent adverse event
TNSS-	Total nasal symptom score
TOSS-	Total ocular symptom score

INTRODUCTION

In Europe and the United States, clinically relevant sensitization to birch pollen affects approximately 20%–30% of the allergic population.¹⁻⁴ Birch pollen is prevalent during tree pollen season, during 4 to 8 weeks of late winter to early summer, depending on the geographic location.⁵ It contains a mix of allergenic and non-allergenic proteins, among which, Bet v 1 is the immunodominant and most abundant allergenic protein.^{6, 7} Sensitization rates to Bet v 1 among birch-allergic individuals are reported to reach >95%.⁶⁻⁸ Allergen sensitivity may result in allergic rhinitis (AR) and allergic conjunctivitis, immunoglobulin E (IgE)-mediated inflammatory disorders. Over 70% of individuals with pollen allergy also experience oral allergy syndrome (OAS) to fresh fruits, vegetables, and nuts due to the presence of structurally similar allergens in pollen and associated foods.^{9, 10} OAS symptoms manifest as itching, swelling and angioedema of lips, mouth and throat.^{11, 12}

AR treatment includes short-term relief of symptoms with antihistamines and intranasal corticosteroids, and allergen immunotherapy (AIT).¹³⁻¹⁶ AIT is used to treat moderate-to-severe AR induced by aeroallergens such as birch and related tree pollens, eg, alder and hazel.¹⁷⁻¹⁹ Immunotherapy using birch pollen extract or Bet v 1 has demonstrated reduction in daily AR symptoms.²⁰ Furthermore, in vitro competition studies using Bet v 1 have shown a high degree of inhibition of human IgE binding to alder, hazel, and oak allergen extracts,²¹ highlighting the presence of similar IgE binding epitopes and potential benefit of birch IT for related tree species. Studies also demonstrate that birch AIT offers symptom amelioration of OAS, although these tended to be small, uncontrolled studies with conflicting results.²²⁻²⁹

Subcutaneous immunotherapy (SCIT) is efficacious, but is limited by the inconvenience of required dosing over months to years, variability in outcomes, and occurrence of adverse events in 40%–50% of patients ranging from mild reactions (eg, swelling, injection-site reaction, *de novo* allergic response, and urticaria) to life-threatening reactions (eg, asthma exacerbation and anaphylaxis).³⁰⁻³² Recent data suggest that allergen-specific blocking of IgG may be a major protective mechanism elicited by AIT, as a cocktail of two Fel d 1-specific blocking IgG antibodies (Fel d 1 is the major allergen in cat dander) was well tolerated and significantly reduced the allergic response in cat-allergic subject almost immediately, with sustained effects up to 12 weeks in some subjects.³³ This novel approach of using passive administration to prevent and treat allergic diseases may potentially overcome several of the limitations associated with available AIT. Blocking mAb cocktails may: (1) be well-tolerated as the allergic subject is not exposed to native allergen; (2) have a fast onset of action, as demonstrated by the proof-of mechanism trial in cat allergy³³; (3) demonstrate reliable efficacy; (4) markedly reduce treatment burden as a single injection may prevent allergic symptoms over the entire birch allergy season; (5) relieve allergic reactions to birch-related pollens and foods; and (6) offer a therapeutic option for patients with uncontrolled asthma for whom AIT is contraindicated, such as those most susceptible to treatment-related systemic reactions³⁴.

To further explore the possibility of passive administration of allergen-blocking IgG as a treatment for allergic diseases, a cocktail consisting of three fully human IgG4 mAbs (REGN5713/14/15) has now been developed against the major birch tree allergen, Bet v 1. The Bet v1 specific mAbs (REGN5713, REGN5714, and REGN5715)

151 were generated using Regeneron's VelocImmune platform.^{35, 36} Pre-clinical studies
152 demonstrated that the three mAbs bind independently and non-competitively to Bet v 1
153 and that all three together provided maximal inhibition of Bet v 1 binding to human
154 polyclonal IgE and potently blocked basophil activation ex-vivo and mast cell
155 degranulation in vivo.(Atanasio et al, in press) Preclinical pharmacokinetic (PK) and
156 pharmacodynamic (PD) data demonstrated that a single 900 mg SC dose of
157 REGN5713/14/15 would provide systemic concentrations for maximal inhibition of mast
158 cell degranulation over the duration of the 2-month birch allergy season. The current
159 study aimed to assess the safety, tolerability, PK, and efficacy of REGN5713/14/15 to
160 reduce the early allergic response to nasal, skin, and basophil responsiveness to
161 challenge with birch and other birch homologous tree allergens.

METHODS

Study design

This randomized, double-blind, placebo-controlled, 2-part phase 1 study enrolled 96 healthy adults: 32 at a single study site (Part A) and 64 with a history of birch-pollen allergy, confirmed by positive skin prick test (SPT) and specific-IgE to birch and Bet v 1 (>0.35 kU/L), at 3 study sites (Part B) in Belgium. In Part A, subjects were randomized 3:1 to REGN5713/14/15, sequential ascending dose cohorts of 150 mg SC (50 mg/antibody), 450 mg SC (150 mg/antibody), 900 mg SC (300 mg/antibody), and 900 mg intravenous (IV) (300 mg/antibody), or placebo, to assess the safety, tolerability, and PK of REGN5713/14/15. In Part B, birch-allergic subjects were randomized 1:1 to receive a single dose of REGN5713/14/15 900 mg SC (300 mg/antibody) or placebo, to determine efficacy in reducing birch pollen-induced AR symptoms (Fig 1), as assessed by nasal symptom response to titrated nasal allergen challenge (NAC) with birch extract and the reduction of early-phase skin responses following SPT with serial birch allergen titration post treatment.

The study was conducted between June 2019 and March 2020. With the exception of the designated study pharmacist(s)/designee at the study site who prepared the study drugs, the study remained blinded to all individuals until the pre-specified unblinding for primary analysis. The study was conducted in accordance with the protocol, the consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines. All study documents were approved

by an Institutional Review Board/Independent Ethics Committee before the study was initiated and all participants signed an informed consent prior to commencing study procedures.

Study population

Subjects aged 18–60 years were eligible, and for Part B, were required to have a history of birch allergy. In Part B, at visit 1 participants were screened for allergen-specific IgE and underwent SPTs with birch allergen extract (ALK-Abelló) and other allergen extracts (alder, hazel, grass, cat, dog, dust mites). Eligibility for further screening in Part B was based on IgE titers specific for Bet v 1 and birch silver IgE >0.35 kU/L each, birch-SPT mean wheal diameter >3mm compared with a negative control SPT, and normal lung function with forced expiratory volume in one second (FEV1) ≥80% predicted. Subjects with a prior history of AIT or vaccination with birch allergen or anti-IgE therapy or AIT to other allergens within 3 months of screening were excluded; for key inclusion and exclusion criteria, see the Supplementary Material. At another screening visit, and if antihistamines were not taken within 5 days of and including the day of NAC, NAC was performed with increasing doses of birch extract (ALK-Abelló), along with a titration SPT to birch and alder. Birch-sensitized patients were eligible for enrollment based on a TNSS <2 prior to the screening NAC, peak TNSS ≥7, and either at least three sneezes or PNIF drop of >20% during up-titration.

203 **Efficacy assessments**

204 ***Nasal allergen challenge***

205 The TNSS (measured on a scale of 0–12) is a composite patient symptom
206 assessment of congestion, itching, and rhinorrhea (each graded on a scale of 0–3, 3
207 being severe), and sneezing (3 being >5 sneezes).³⁷ The 4-item total ocular symptom
208 score (TOSS) (each measured on a scale of 0–3, 3 being severe, total score range 0–
209 12) is a composite symptom assessment of ocular symptoms (itching/burning, redness,
210 watering, tearing, and puffiness and swelling).³⁸ The peak nasal inspiratory flow (PNIF,
211 L/min) is a measure of nasal patency or nasal congestion. At the screening NAC visit,
212 TNSS, TOSS and PNIF were measured pre-NAC and during NAC; for details, see the
213 Methods section in this article's Online Repository.

214 Eligible study participants were randomized to receive study drug or placebo on
215 study day 1, at least 14 days after the NAC screening visit. On study days 8, 29, and 57,
216 study participants underwent NAC, repeating all steps, including placebo challenge and
217 using the same allergen titration required for each individual subject to reach TNSS ≥ 7
218 at their NAC screening visit, not to exceed the maximum dose/qualifying dose
219 established at the screening visit.

220 ***Skin prick test***

221 SPT with serial allergen titration to birch allergen (Allergen source: ALK- Abelló) and
222 one other birch homologous tree allergen, eg, alder (Allergen source: HAL Allergy) was
223 performed on each of the subjects forearms (one allergen type per forearm) at the
224 screening visit and on study days 8, 29, 57, and 113.

Basophil activity test

The basophil activation test (BAT) was performed at a single center at University of Ghent during Part B of the study in 26 study subjects (REGN5713/14/15, n=13; placebo, n=13). BATs were performed using Bühlmann's Flow CAST[®] kits (Bühlmann, Laboratories AG, Switzerland), in which CCR3 and CD63 were used for basophil detection following stimulation of basophils in patients' whole blood *ex vivo* with seven increasing concentrations of allergen extract (0.0055–22.72 ng/mL) to generate a dose-response curve. Basophil activation was measured by the percentage of basophils positive for CD63 surface expression in flow cytometry analysis. Basophil responsiveness to birch, alder, hazel, apple, and grass mix (Allergen source: Bühlmann Laboratories AG, Switzerland or Biomay AG, Vienna) extract was measured by EC50, the allergen concentration required to achieve 50% of maximal basophil activation. A higher EC50 value indicates lower basophil responsiveness to allergen stimulation.

Endpoints and statistical analysis

For Part A, subject disposition, demographics, and baseline characteristics, and safety and tolerability, including incidence of treatment-emergent adverse events (TEAEs), were assessed. The concentration time profiles and PK parameters following a single dose of REGN5713/14/15 were determined from serum samples taken during the Phase 1 study and concentration time profiles were plotted. The concentrations of REGN5713/5714/5715 in serum were used to calculate the PK parameters for the three individual antibodies by non-compartmental analysis using Phoenix WinNonlin v6.4.

The primary efficacy endpoint in Part B was the change in area under the curve (AUC) for TNSS over the first hour (0–1hr) from the screening (pretreatment) NAC challenge to the day 8 challenge. For the SPT with birch (and alder) titration, the AUC of the average of two mean wheal diameters was calculated over the birch (or alder) allergen concentrations on a log scale. For further details, see this article's Online Repository.

Secondary efficacy endpoints change and percent change from pre-treatment baseline TNSS, TOSS, and PNIF AUC (0 to 1 hour after qualifying dose) in response to a NAC, and SPT AUC, were analyzed by mixed-effect model repeated measures (MMRM). Between-group estimates comparing REGN5713/14/15 with placebo, and confidence intervals and nominal *P*-values were reported for NACs/SPTs on days 8, 29, and 57 (and 113 for SPT).

RESULTS

Study participants

Overall, 32 healthy adults were enrolled in Part A and 64 healthy adults with a history of birch-pollen allergy were enrolled in Part B. Baseline demographics and characteristics of subjects in Part B, including age, sex, race, ethnicity, and body mass index, were generally similar across treatment groups (Table 1). A summary of baseline responses to birch SPT and IgE levels is provided in Table 1; these were all relatively balanced across treatment groups. Baseline IgE and SPT responses to other common allergens is shown in Table E1 and Table E2, respectively. During the course of the study, three placebo recipients (9.4%) and one REGN5713/5714/5715 recipient (3.1%) used antihistamines; however, none were used during the concomitant medication use exclusion window prior to a NAC.

A single dose of REGN5713/14/15 reduced TNSS AUC(0–1h) after NAC

Baseline TNSS measured between 0 and 1 hours after the baseline NAC qualifying dose was similar between treatment groups, with a similar AUC(0–1 hr) of TNSS following the baseline NAC, 4.1 and 4.1 in the REGN5713/14/15 and placebo groups, respectively (Table 1).

Compared with placebo, single-dose REGN5713/14/15 significantly reduced TNSS AUC(0–1 hr) following NAC on day 8, 29, and 57, placebo-adjusted changes in TNSS AUC(0–1 hr) from the baseline NAC, -1.2 ($P = .001$), -1.2 ($P = .001$), and -0.9 ($P = .024$), respectively (Fig 2). The percent change from baseline yielded similar results, with significant reductions in TNSS AUC(0–1 hr) compared with the baseline

NAC up to day 57; placebo-adjusted changes from baseline NAC were -31.6% ($P = .002$), -27.4% ($P = .003$), and -19.0% ($P = .053$) on days 8, 29, and 57, respectively (Fig 2, A). The decrease in relative efficacy of REGN5713/14/15 on day 57 was associated with an increase in the placebo effect, percent change in TNSS AUC(0–1 hr) of -31.4%, -31.2%, and -42.0% on days 8, 29, and 57, respectively. The treatment effect of REGN5713/14/15 remained at about 60% across all study days: TNSS AUC(0–1 hr) -63.0% (day 8), -58.5% (day 29), and -61.0% (day 57).

Total ocular symptoms and nasal airflow

At baseline, subjects' NAC-provoked ocular symptoms were minimal; mean TOSS AUC 1.3 and 2.1 in the REGN5713/14/15 and placebo groups, respectively. Compared with placebo, REGN5713/14/15 significantly reduced TOSS AUC(0–1 hr) following NAC at 8 and 29 days after a single dose, with a trend for reduction on day 57, placebo-adjusted changes in TOSS AUC from baseline NAC on days 8, 29, and 57 were -41.6% ($P = .003$), -48.3% ($P = .022$) and -36.6% ($P = .178$), respectively (Fig 2, B).

PNIF AUC increased from baseline (REGN5713/14/15, 59.9; placebo 53.1) by more than 80% for subjects 8, 29, and 57 days after receiving a single dose of REGN5713/14/15; placebo-adjusted percent changes in PNIF AUC from the baseline NAC were 32.2% [$P = .092$], 47.1% [$P = .017$], and 34.8% [$P = .121$], respectively, with statistical significance versus placebo only reached on day 29 (Fig 2, C).

Titration skin prick test

Mean wheal diameters in the serial birch titration SPT at the baseline visit were similar between the REGN5713/14/15 and placebo groups; AUC of mean wheal

diameters were 4.1 and 3.9, respectively, (Table 1). REGN5713/14/15 significantly reduced the AUC of the SPT mean wheal diameters (Fig 3) relative to placebo out to day 113. Placebo-adjusted percent changes relative to the baseline SPT were: -72.4% ($P < .001$), -65.6% ($P < .001$), -77.9% ($P < .001$), and -73.6% ($P < .001$) on study days 8, 29, 57, and 113, respectively, (Fig 3, A).

AUC of mean wheal diameters of the serial alder titration SPT at baseline also were similar between the REGN5713/14/15 (5.3) and placebo groups (4.9) (Table 1). REGN5713/14/15 significantly reduced the AUC of alder SPT mean wheal diameters (Fig 3) relative to placebo out to day 113, placebo-adjusted percent changes relative to the baseline SPT were: -44.7% (day 8, $P < .001$), -46.3% (day 29, $P < .001$), -52.8% (day 57, $P < .001$), -49.8% (day 113, $P < .001$) (Fig 3, B).

Correlation between TNSS and SPT clinical outcomes

After a single dose of REGN5713/14/15, a significant positive correlation was observed between TNSS response, as measured by percent change from baseline TNSS AUC(0–1 hr), and SPT response, as measured by percent change from baseline in SPT AUC, on days 8 ($P < .001$), 29 ($P < .01$), and 57 ($P < .0001$) (Fig E1).

Correlation of baseline IgE with clinical outcomes

No significant correlation was observed between Bet v 1 and Birch Silver IgE at baseline with TNSS response (Fig E2). Baseline Bet v 1 IgE and Birch Silver IgE were weakly correlated with SPT response on day 8 after dosing in subjects who received REGN5713/14/15 (Fig E3).

Reduced basophil responsiveness to birch pollen, hazel, alder and Mal d 1 (apple) after treatment with REGN5713/14/15

REGN5713/14/15 significantly suppressed basophil responsiveness to birch pollen extract versus placebo, as measured by EC50 (days 8, 57, and 113, all $P < .01$, Fig E5) and percent change from baseline in EC50 (days 8, 57, and 113, all $P < .0001$, Fig 4). In the REGN5713/14/15 group, the highest EC50 was detected on day 8 with the inhibitory effect maintained up to day 113. As assessed by percent change in EC50 from baseline, basophil responsiveness to alder, hazel pollen extract and recombinant Mal d 1 was significantly suppressed post REGN5713/14/15 treatment versus placebo (alder: days 8, 57, 113 [all $P < .001$]; hazel: day 8, $P < .001$; day 57, $P = .01$; Mal d 1: day 8, $P < .01$; days 57 and 113, $P < .05$). No suppression in basophil response to grass allergen mix (negative control) was detected with REGN5713/14/15 treatment (Fig 4, A-E). These data are consistent with our *in vitro* binding data demonstrating cross reactivity of the Bet v 1 mAbs to structurally related allergens including rAln g 1 (Alder), rMal d 1 (apple) and rCor a 1 (Hazel); REGN5713, REGN5714 and REGN5715 were shown to bind to homologous allergens in Alder, Hazel and hornbeam, while REGN5715 also bound to those in apple (Atanasio et al. in press).

For the REGN5713/14/15 treatment group, there was an inverse correlation between the percent change in TNSS AUC(0–1hr) and EC50 for birch allergen on day 8 ($r = -0.71$; $P = .0095$; Fig 5, A), and inverse correlations between birch EC50 and the percent change in birch SPT (mean wheal diameter) AUC on day 8 ($r = -0.6$; $P = .04$) and day 57 ($r = -0.57$; $P = .04$; Fig 5, B). A similar relationship was observed in a subgroup analysis, where the subjects with $\geq 60\%$ mean reduction in TNSS AUC(0-1hr)

(Fig E4, A) and birch SPT (mean wheal diameter AUC) (Fig E4, B) tended to have higher birch BAT EC50.

Pharmacokinetics

The concentration time profiles and PK parameters of REGN5713/14/15 in healthy subjects exhibited no signs of concentration dependence, consistent with linear PK. The dose normalized AUC_{∞} was similar at each SC dose level for all mAbs and the C_{max} increased proportionally with dose. The three individual mAbs showed half-lives ranging from 25–36 days. Bioavailability for the three individual mAbs ranged from 70–93%. Comparison of the concentration time profiles in Part A and Part B showed virtually no difference in PK between healthy subjects and Bet v 1-sensitive subjects (Fig 6).

A single dose of REGN5713/14/15 was well tolerated

No serious adverse events were reported in Part A or B of the study, and no deaths or TEAEs led to study discontinuation (Table E3, Table E4). The overall frequency of TEAEs up to 16 weeks was comparable following treatment with REGN5713/14/15 SC (Part A: 88.9%, Part B: 81.3%) and placebo (Part A: 100%, Part B: 93.8%). No dose-dependent trend in the incidence of any TEAEs was observed. The overall frequency of treatment-related TEAEs was also comparable between REGN5713/14/15 (Part A: 25.0%, Part B: 21.9%) and placebo (Part A: 12.5%, Part B: 25.0 %). There were a total of 13 injection site reactions in this study, all of which were mild in severity. In Part A, 2 injection-site reactions were reported (1 [16.7%] each in the REGN5713/14/15 450 mg SC group and the 900 mg SC group. In Part B, 11 injection-site reactions were reported (5 [15.6%] in the REGN5713/14/15 group and 6 [18.8%] in the placebo group. There

was a total of 8 hypersensitivity reactions in this study. In Part A, 1 non-serious TEAE of rash met the hypersensitivity criteria in a subject who received 900 mg IV REGN5713/14/15. The rash, which occurred above the left eye on study day 45, was mild in severity, deemed not related to study drug, and resolved without treatment in a subject with a history of ocular eczema. In Part B, 3 TEAEs met the hypersensitivity criteria in 3 (9.4%) subjects receiving REGN5713/14/15 as compared to 4 TEAEs in 3 (9.4%) subjects who received placebo. In subjects receiving REGN5713/14/15, 1 subject each experienced oral allergy syndrome (1.6%), contact dermatitis (1.6%), and urticaria (1.6%). These 3 TEAEs were mild and only the oral allergy syndrome event was related to study drug. No dose-dependent trend in any of the treatment-related TEAEs was observed.

DISCUSSION

In this phase 1 study in healthy and birch-sensitized allergic subjects, compared with placebo, a single SC dose (900 mg) of REGN5713/14/15 significantly inhibited nasal symptoms in response to birch allergen NAC as early as 1-week post treatment. Clinically meaningful nasal symptom reduction after NAC was maintained for 8 weeks, the last timepoint that was tested and the approximate duration of a typical birch pollen season, although this can be prolonged if pollen from several *Fagales* tree species is present.^{39, 40} SPT responses to birch allergen were significantly reduced in subjects treated with REGN5713/14/15 up to 16 weeks after dosing, at the last timepoint that was tested. The tolerability and safety profile of REGN5713/14/15 was comparable with that of placebo. These clinical data support the previously published proof-of-mechanism study³³, where passive administration with a single dose of a Fel d 1 mAb cocktail effectively reduced cat-allergen induced AR symptoms.

Birch pollen allergy poses a substantial clinical impact in birch-sensitive subjects during tree pollen season, including AR and conjunctivitis,⁴¹ worsening of asthma symptoms in those with comorbid asthma, and reduced quality of life, and work and school productivity measures.¹⁵ Treatment of birch allergy is limited to symptomatic control with antihistamines and intra-nasal steroids, which may be ineffective in approximately half of patients,^{14, 15} and AIT, for which patient adherence can be low due to the inconvenience of dosing and safety concerns.^{42, 43} Limited available data suggest that compared with pharmacotherapy, the effects of AIT take longer to develop, but once established, achieve longer-lasting relief of allergic symptoms, while the benefits of pharmacotherapy only last as long as treatment is continued.³¹ Together with the

401 results from the proof-of-mechanism study using a cocktail of cat-allergen-specific IgG
402 antibodies³³, our findings support the notion that allergen-specific IgG may be a major
403 protective mechanism elicited by AIT, and that passive administration of recombinant
404 allergen-specific IgG mAb cocktails may provide for a well-tolerated, fast and
405 convenient treatment approach for many allergies.

406 In this study, compared with placebo, a single SC dose of REGN5713/14/15
407 significantly reduced nasal symptoms at birch allergen challenge as early as 1 week,
408 and subsequently at 1 month and 2 months. Additionally, REGN5713/14/15 significantly
409 reduced skin test reactivity (wheal diameter) provoked by a SPT with serial birch
410 allergen titration at these time points and at 4 months. These data corroborate with the
411 PK data, where the combined concentration of REGN5713/14/15 was maintained at
412 approximately 10 mg/L on day 113, which is longer than the expected birch pollen
413 season that is typically 4–12 weeks,⁵ depending on the geographic location. However,
414 as the birch pollen season may be longer than the current study duration, field studies
415 are needed to confirm the durability of a single dose. The REGN5713/14/15 10 mg/L
416 concentration showed total blockade of mast cell degranulation in the Passive
417 Cutaneous Anaphylaxis (PCA) mouse model. This concentration of sIgG is higher than
418 the levels of sIgG achievable after long-term birch AIT⁴⁴; however, the PK and efficacy
419 of REGN5713/14/15 will need to be evaluated in a field study during the birch allergy
420 season to determine whether these drug concentrations are maintained over time in the
421 presence of persistent environmental birch pollen.

422 We used basophil responsiveness to assess the effect of this approach on effector
423 cell activation, because of the relative accessibility of basophils in circulation.

Importantly, the utility of BATs in clinical research applications, such as studying immune mechanisms of allergic disease, is increasingly recognized.⁴⁵⁻⁴⁷ The upregulation of CD63+, the lysosome marker, to the cell surface is used as a marker for basophil activation and degranulation. This is a key step in the inflammatory cascade that triggers allergic responses, hence can be used to investigate potential effectors of the immune response.⁴⁵ We observed that treatment with REGN5713/14/15, but not placebo, significantly lowered basophil response to birch pollen extract. Notably, the EC50 for birch allergen correlated with improvements in clinical responses, indicating that the less sensitive the basophils are to birch pollen (i.e., higher EC50), the greater the clinical benefit the patients may achieve. Thus, it appears that the inhibition of basophil/mast cell degranulation may be a key mechanism by which REGN5713/14/15 ameliorated the allergic symptoms in this study.

Previous studies have shown that pre-incubation with the major birch allergen, Bet v 1 results in a high degree of inhibition of human IgE binding to alder, hazel, and oak allergen extracts.²¹ In agreement with this, we observe cross-reactive binding of our Bet v 1 mAbs to structurally similar allergens from alder and hazel, as well as the major apple allergen, Mal d 1.(Atanasio et al, in press) Following treatment with REGN5713/14/15, there was significant suppression of basophil responsiveness to Alder (r Alng 1), Hazel (r Cor a 1) pollen extract, and Apple (r Mal d 1). These data are also consistent with the SPT results which showed that REGN5713/14/15 significantly reduced mean wheal diameter after SPT with alder pollen extract. The inhibitory effect on basophil responses to homologous allergens suggests that REGN5713/14/15 as a cocktail may ameliorate allergic response to related tree allergens beyond birch and

may treat birch pollen related OAS. Future studies are needed, using double-blind food challenge, to confirm the efficacy of REGN5713/14/15 for the treatment of OAS.

In conclusion, based on this Phase 1 nasal provocation study, a single SC dose of REGN5713/14/15 was well tolerated and provided a rapid and durable reduction in allergic symptoms post birch-allergen provocation. The suppression of the allergic response to other cross-reacting allergens by the novel Bet v 1 mAb cocktail may offer a therapy for the prevention and treatment of seasonal birch and related allergies with rapid onset at 1 week and sustained over 2 months. The 3-mAb cocktail targeting the dominant birch allergen, Bet v 1, provided substantial clinical improvement in most patients. Of note, one patient with OAS attempted to eat apples and was elated to find that consumption of apples was tolerable for several weeks, a finding that opens perspectives for further clinical trials. This study has laid the foundation for future field studies to evaluate the clinical efficacy of REGN5713/14/15, and the optimal dosing schedule, in treating birch pollen allergy in the real-world setting. Moreover, our findings support the possibility that passive administration of recombinant allergen-specific IgG cocktails may provide for a well-tolerated, faster and more convenient treatment paradigm for many allergies.

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Table 1. Demographic and baseline characteristics of birch-allergic study participants

Mean (SD)*	REGN5713/14/15 900 mg SC (n = 32)	Placebo total SC (n = 32)	Total (N = 64)
Age, years	33.8 (11.2)	39.9 (11.4)	36.9 (11.6)
Gender, male, n (%)	9 (28.1)	13 (40.6)	22 (34.4)
Ethnicity, n (%)			
Not Hispanic or Latino	32 (100)	32 (100)	64 (100)
Race, n (%)			
White	31 (96.9)	32 (100)	63 (98.4)
Body mass index, kg/m ²	24.2 (3.2)	24.6 (3.4)	24.4 (3.3)
Baseline allergy measures and IgE levels			
AUC of TNSS (0–1 hr)	4.1 (1.6)	4.1 (1.4)	4.1 (1.5)
Peak TNSS (0–1 hr)	8.0 (1.0)	7.7 (0.8)	7.8 (0.9)
AUC of TOSS (0–1 hr)	1.3 (1.2)	2.1 (2.2)	1.7 (1.8)
AUC of PNIF (0–1 hr)	59.9 (32.2)	53.1 (27.9)	56.5 (30.1)
Screening SPT for birch allergen (mm)	6.5 (1.3)	6.8 (2.0)	6.7 (1.7)
AUC of SPT with serial birch allergen titration	4.1 (1.4)	3.9 (1.3)	4.0 (1.3)
AUC of SPT with serial alder allergen titration	5.3 (1.5)	4.9 (1.5)	5.1 (1.5)
Bet v 1 IgE, median (range)	15.3 (0.6 : 99.5)	8.5 (0.7 : 101.0)	12.0 (0.6 : 101.0)
Birch Silver IgE, median (range)	19.1 (0.8 : 101.0)	10.1 (0.7 : 101.0)	13.6 (0.7 : 101.0)
Bet v 2 IgE, median (range)	0.05 (0.05 : 11.3)	0.05 (0.05 : 9.1)	0.05 (0.05 : 11.3)
Total IgE, median (range)	126.0 (28.0 : 1120.0)	48.0 (5.0 : 1301.0)	87.5 (5.0 : 1301.0)

*Unless otherwise specified.

AUC, area under the curve; IgE, immunoglobulin E; N/n, number; SD, standard deviation; SPT, skin prick test; TNSS, total nasal symptom score.

FIG 1. Study design including Part A in healthy volunteers and Part B, a proof-of-concept arm, in birch-allergic healthy subjects.

BAT, Basophil activation test; *FIH*, first-in-human; *FPFV*, first participant, first visit; *LPLV*, last participant, last visit; *NAC*, nasal allergen challenge; *SPT*, skin prick test.

FIG 2. A single dose of REGN5713/14/15 900 mg SC (**A**) reduces % change from baseline TNSS AUC(0–1 hr); (**B**) reduces % change from baseline TOSS AUC(0–1 hr); and (**C**) improves % change from baseline PNIF AUC(0–1 hr) after NAC day 8, 29, and 57 post dosing versus placebo; LS mean % change from baseline NAC AUC, adjusted for baseline AUC (and treatment-by-baseline interaction for TOSS); LS mean change (LS mean percent change) from baseline NAC AUC is presented for placebo and REGN5713/14/15 900 mg SC in the table, adjusted for baseline AUC. Mean (SD) baseline values of each measure are presented for day 0. SEs for these LS mean estimates are provided in **Table E5**.

* $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$.

AUC, area under the curve; *LS*, least squares; *NAC*, nasal allergen challenge; *PNIF*, Peak nasal inspiratory flow; *SC*, subcutaneous; *SE*, standard error; *TNSS*, total nasal symptom score; *TOSS*, total ocular symptom score.

Fig 3. A single dose of REGN5713/14/15 900 mg SC reduces % change from baseline SPT AUC for birch (**A**) and alder (**B**) at day 8, 29, 57 and 113 after dosing versus placebo, and reduces LS mean (SE) change from baseline SPT AUC (presented in the table), adjusted for baseline AUC.

***All study days: $P < .001$.

AUC, area under the curve; *LS*, least squares; *SC*, subcutaneous; *SE*, standard error; *SPT*, skin prick test.

FIG 4. The percent change in EC50 from baseline showed significant suppression of basophil responsiveness to birch (**A**), alder (**B**), hazel (**C**) and apple (**D**) pollen extract following treatment with REGN5713/14/15 versus placebo. No suppression of basophil responsiveness to the negative control, grass pollen extract (**E**), was detected. Several large values were excluded in the display for apple and grass; all P -values presented consider all data points based on non-parametric Wilcoxon test.

EC50, the allergen concentration required to achieve 50% of maximal basophil activation; *SC*, subcutaneous. * $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$, **** $P \leq .0001$.

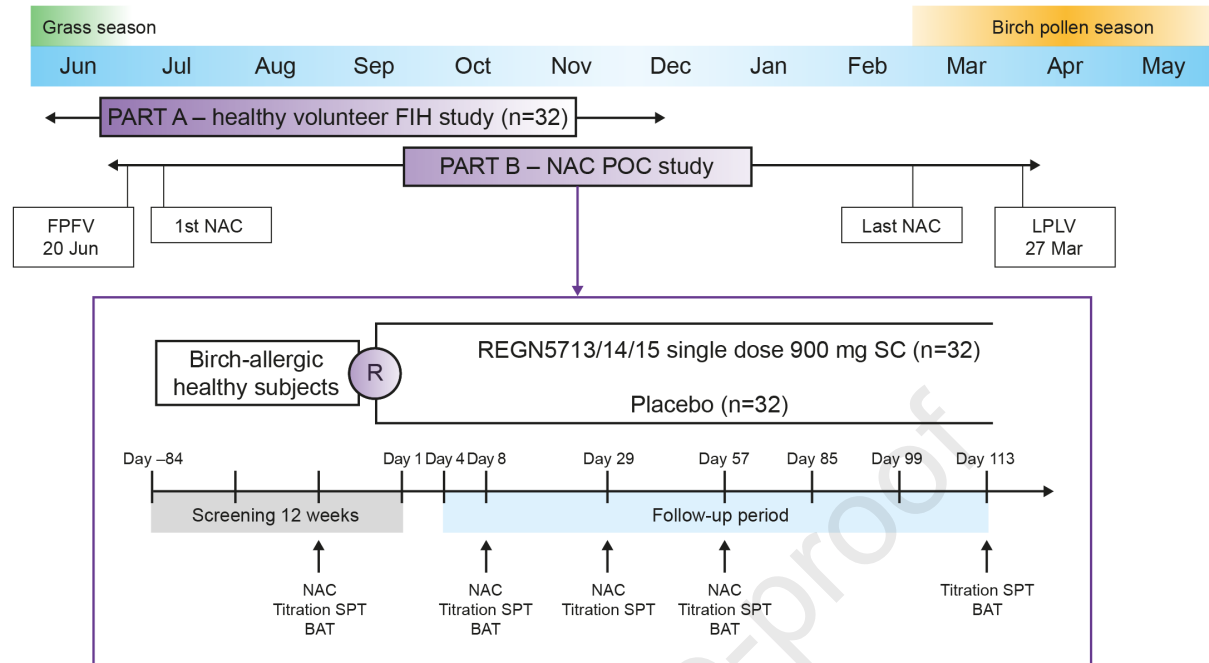
FIG 5. Correlation between basophil response and clinical symptom improvement for TNSS and for SPT showed: (**A**) inverse correlation between the percent change in TNSS AUC(0–1hr) and EC50 for birch allergen on day 8 ($r = -0.71$; $P = 0.0095$) and (**B**) inverse correlations between birch BAT EC50 and the percent change in birch SPT (mean wheal diameter) AUC on day 8 in ($r = -0.6$; $P = .04$) and day 57 ($r = -0.57$; $P = .04$)

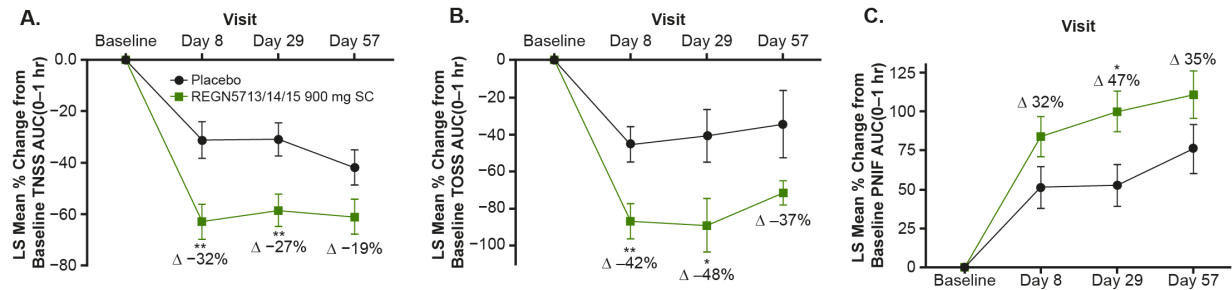
653 in subjects who received REGN5713/14/15; no significant correlation was observed in
654 subjects who received placebo.

655 *AUC*, area under the curve; *BAT*, basophil activation test; *EC50*, the allergen concentration required to achieve 50%
656 of maximal basophil activation; *NAC*, nasal allergen challenge; *PNIF*, Peak nasal inspiratory flow; *SPT*; skin prick
657 test; *TNSS*, total nasal symptom score.

658 **FIG 6.** Mean concentration of total REGN5713, REGN5714, and REGN5715 following a
659 single SC dose (900 mg) in Bet v 1-sensitive patients is not significantly different from
660 that seen in healthy subjects.

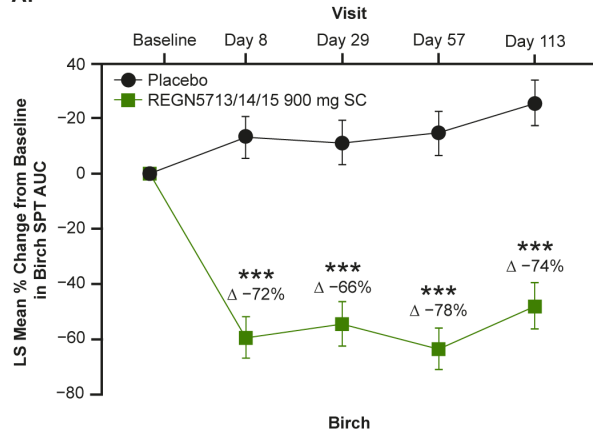
661 SC, subcutaneous.



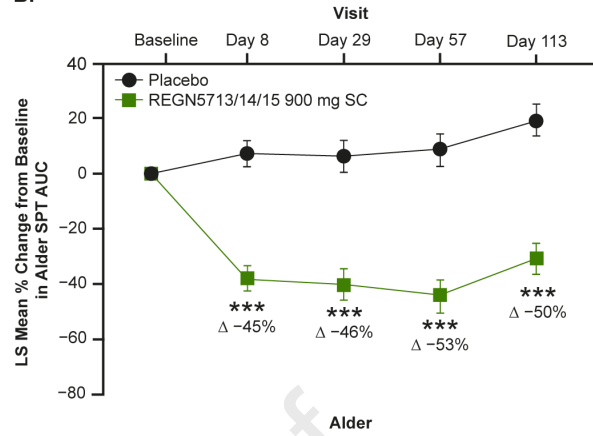


Measure	Study Day	Placebo, n	REGN5713/14/15, n	Placebo Mean Change (% Change)	REGN5713/14/15 Mean Change (% Change)	Placebo vs. REGN5713/14/15 Mean Difference (% Difference)	P-value (Placebo vs. REGN5713/14/15, Mean Change (% Change))
TNSS	0	30	32	4.13 (1.45)	4.05 (1.58)		
	8	30	32	-1.50 (-31.39)	-2.67 (-62.96)	-1.17 (-31.57)	.001 (.002)
	29	29	30	-1.39 (-31.15)	-2.57 (-58.50)	-1.18 (-27.35)	.001 (.003)
	57	30	28	-1.79 (-41.96)	-2.64 (-60.96)	-0.85 (-19.00)	.024 (.053)
TOSS	0	30	32	2.13 (2.16)	1.25 (1.19)		
	8	30	32	-0.88 (-45.30)	-1.36 (-86.88)	-0.48 (-41.58)	.013 (.003)
	29	29	30	-0.85 (-40.82)	-1.44 (-89.09)	-0.59 (-48.27)	.007 (.022)
	57	30	28	-0.99 (-34.85)	-1.29 (-71.47)	-0.30 (-36.62)	.191 (.178)
PNIF	0	30	32	53.14 (27.91)	59.92 (32.20)		
	8	30	32	22.07 (51.42)	38.43 (83.64)	16.45 (32.22)	.065 (.092)
	29	29	30	22.37 (52.59)	47.65 (99.69)	25.28 (47.10)	.007 (.017)
	57	30	28	33.58 (75.88)	50.32 (110.70)	16.74 (34.83)	.078 (.121)

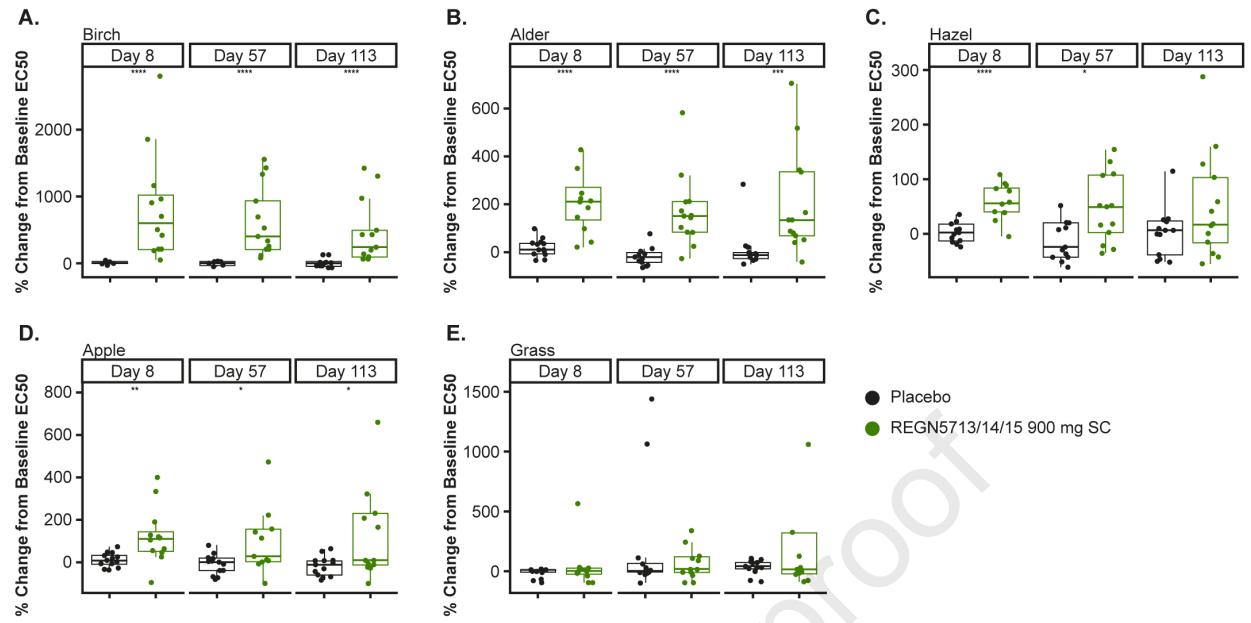
A.



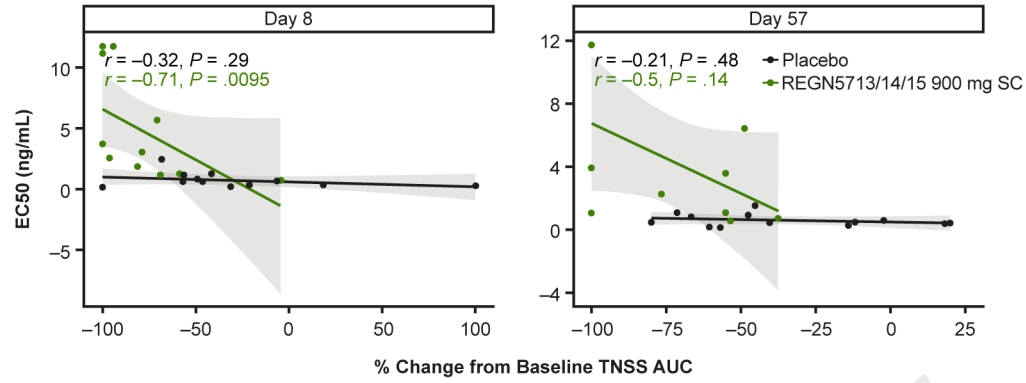
B.



Allergen for Titration SPT	Study Day	N Placebo	N REGN5713/14/15 900 mg SC	Mean (SE) Change, Placebo	Mean (SE) Change, REGN5713/14/15 900 mg SC	Mean (SE) Difference (Placebo vs REGN5713/14/15 900 mg SC)	P-value (Placebo vs REGN5713/14/15 900 mg SC)
Birch	Day 8	32	32	0.11 (0.24)	-2.43 (0.24)	-2.54 (0.35)	< .001
	Day 29	32	32	0.00 (0.25)	-2.27 (0.25)	-2.27 (0.36)	< .001
	Day 57	32	32	0.21 (0.27)	-2.57 (0.27)	-2.78 (0.39)	< .001
	Day 113	32	32	0.50 (0.26)	-1.97 (0.26)	-2.46 (0.37)	< .001
Alder	Day 8	32	32	0.11 (0.23)	-2.01 (0.23)	-2.12 (0.33)	< .001
	Day 29	32	32	0.02 (0.26)	-2.08 (0.26)	-2.10 (0.36)	< .001
	Day 57	31	32	0.16 (0.30)	-2.26 (0.30)	-2.42 (0.42)	< .001
	Day 113	32	32	0.69 (0.26)	-1.74 (0.26)	-2.43 (0.37)	< .001



A.



B.

