Oral once-daily berotralstat for the prevention of hereditary angioedema attacks: A randomized, double-blind, placebo-controlled phase 3 trial

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Background: Berotralstat (BCX7353) is an oral, once-daily inhibitor of plasma kallikrein in development for the prophylaxis of hereditary angioedema (HAE) attacks.
Objective: Our aim was to determine the efficacy, safety, and tolerability of berotralstat in patients with HAE over a 24-week treatment period (the phase 3 APeX-2 trial).
Methods: APeX-2 was a double-blind, parallel-group study that randomized patients at 40 sites in 11 countries 1:1:1 to receive once-daily berotralstat in a dose of 110 mg or 150 mg or placebo (Clinicaltrials.gov identifier NCT03485911). Patients aged 12 years or older with HAE due to C1 inhibitor deficiency and at least 2 investigator-confirmed HAE attacks in the first 56 days of a prospective run-in period were eligible. The primary efficacy end point was the rate of investigator-confirmed HAE attacks during the 24-week treatment period.
Results: A total of 121 patients were randomized; 120 of them received at least 1 dose of the study drug (n = 41, 40, and 39 in the 110-mg dose of berotralstat, 150-mg of dose berotralstat, and placebo groups, respectively). Berotralstat demonstrated a significant reduction in attack rate at both 110 mg (1.65 attacks per month; $P = .024$) and 150 mg (1.31 attacks per month; $P < .001$) relative to placebo (2.35 attacks per month). The most frequent treatment-emergent adverse events that occurred more with berotralstat than with placebo were abdominal pain, vomiting, diarrhea, and back pain. No drug-related serious treatment-emergent adverse events occurred.
Conclusion: Both the 110-mg and 150-mg doses of berotralstat reduced HAE attack rates compared with placebo and were safe and generally well tolerated. The most favorable benefit-to-risk profile was observed at a dose of 150 mg per day. (J Allergy Clin Immunol 2020;∎∎∎:∎∎∎-∎∎∎.)

Key words: BCX7353, berotralstat, C1 inhibitor, efficacy, HAE, hereditary angioedema, kallikrein inhibitor, long-term prophylaxis, prophylaxis, safety

Hereditary angioedema (HAE) due to C1 inhibitor (C1-INH) deficiency (HAE–C1-INH) is an autosomal-dominant disorder resulting from mutations in the *SERPING1* gene.[1,2] Reduced

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Abbreviations used

AE:	Adverse event
AE-QoL:	Angioedema Quality of Life Questionnaire
C1-INH:	C1 inhibitor
HAE:	Hereditary angioedema
HAE–C1-INH:	Hereditary angioedema caused by C1 inhibitor deficiency
LLN:	Lower limit of normal
MCID:	Minimal clinically important difference
LSM:	Least squares mean
OR:	Odds ratio
QoL:	Quality of life
SOC:	Standard of care
TEAE:	Treatment-emergent adverse event
TESAE:	Treatment-emergent serious adverse event
TSQM:	Treatment Satisfaction Questionnaire for Medication

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functional levels of C1-INH, the primary inhibitor of the contact system proteases plasma kallikrein and activated coagulation factor XII, result in dysregulation of the contact system with excess generation of bradykinin, the primary mediator of swelling in HAE–C1-INH.[3,4]

HAE–C1-INH is a rare disorder characterized by recurrent episodes of subcutaneous or mucosal angioedema.[1] HAE attacks typically first occur during childhood and recur with variable severity and frequency throughout life.[1] These attacks are unpredictable, often associated with significant morbidity, and potentially fatal as a result of asphyxiation due to laryngeal angioedema.[5] The mean frequency of attacks in untreated patients is approximately every 2 weeks,[6] with individual attacks lasting 3 to 5 days before fully resolving.[7] The combination of asphyxiation risk, unpredictability, severity, and frequency of attacks justify prophylactic treatment; the possibility of passing HAE–C1-INH to the next generation contributes to a severe disease burden with markedly reduced quality of life (QoL).[8,9]

Treatment options for HAE–C1-INH have undergone a remarkable transformation in the past decade.[10] Previously, HAE attacks were predominantly managed with symptomatic treatment that maintained the airway and relieved pain, nausea, and vomiting but did not shorten the duration of symptoms.[1] Prophylactic treatment to reduce the frequency and severity of attacks had consisted primarily of 17-α-alkylated (anabolic) androgens, which are effective but could require high doses,[11] with dose-dependent side effects that markedly limit their tolerability and present challenges for use in women and children.[12] Elucidation of the underlying pathophysiology of HAE–C1-INH has led to the development and approval of multiple effective medications that can treat or prevent HAE attacks.[13,14] These medications fall into 3 mechanistic groups: intravenous or

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subcutaneous C1-INH replacement, subcutaneous inhibitors of plasma kallikrein, or a subcutaneous bradykinin B$_2$ receptor antagonist. These mechanism-based therapies have substantially improved symptom control and QoL for patients with HAE–C1-INH; however, all require parenteral administration.

Berotralstat (BCX7353) is an oral, highly specific plasma kallikrein inhibitor with a pharmacokinetic profile that supports once-daily dosing.[15] Here, we report the 24-week efficacy and safety results of an ongoing phase 3 trial of berotralstat for the long-term prophylactic treatment of patients with HAE–C1-INH.

METHODS
Study design

The APeX-2 (BCX7353-302) trial was a phase 3, randomized, double-blind, placebo-controlled, parallel-group multicenter trial conducted at 40 sites in 11 countries (ClinicalTrials.gov: NCT03485911 [see Table E1 in the Online Repository at www.jacionline.org]). Part 1 of the trial, which is reported here, was a 24-week, double-blind evaluation of the efficacy of prophylactic berotralstat, 110 mg and 150 mg, administered orally once daily and compared with placebo. Patients were randomized 1:1:1 to receive berotralstat, 110 mg or 150 mg, or placebo administered orally once daily; randomization was stratified by baseline attack rate (≥2 vs <2 attacks per month [see Fig E1 in the Online Repository at www.jacionline.org]). The 1:1:1 randomization (block size of 6) was generated by using SAS software (SAS Institute Inc, Cary, NC) and uploaded to the interactive web response system (Veracity Logic, Chapel Hill, NC). All patients, investigators, and site and sponsor personnel were blinded to treatment group allocation, except for sponsor or vendor staff responsible for the management of study drug supplies. Part 1 of the study is complete; however, later parts of this study remain ongoing.

Patients

Patients with HAE–C1-INH were eligible if aged 12 years or older if living in the United States and Canada and 18 years or older if living in Europe. The diagnosis of HAE–C1-INH was confirmed by C1-INH functional level (<50% by chromogenic assay) and complement 4 level less than the lower limit of normal (LLN). Patients with a C1-INH functional level between 50% and the assay LLN (74%) or a complement 4 value greater than the LLN could qualify for inclusion under additional alternative protocol–specified criteria. These alternative criteria are detailed in the Methods section of this article's Online Repository (at www.jacionline.org). A prospective run-in period of up to 70 days was used to determine baseline attack rate. Patients with 2 or more distinct investigator-confirmed HAE attacks requiring treatment or causing functional impairment in the first 56 days of the prospective run-in period were eligible for enrollment. Enrolled patients were required to have access to at least 1 approved standard of care (SOC) on-demand medication to treat HAE attacks; treatment of attacks followed the patients' usual medical management plan. Patients were excluded if they had used androgen or tranexamic acid prophylaxis within 28 days of screening or C1-INH prophylaxis within 14 days of screening.

Before initiation of the study, all patients or caregivers provided written informed consent and assent as appropriate. Additional study documentation, including protocols and patient information sheets, were submitted to institutional review boards and independent ethics committees for approval. This study was conducted in compliance with the current revision of the Declaration of Helsinki and current International Council for Harmonization and Good Clinical Practice guidelines. An independent data monitoring committee provided review of safety data at prespecified intervals, with additional consultation or review as needed.

Procedures

Patients recorded the frequency, duration, location, functional impact, and any treatment of HAE attacks experienced in the previous 24 hours in an electronic diary daily. Investigators contacted patients within 2 business days of each reported attack to discuss and evaluate the event. All investigator-confirmed attacks required a symptom of swelling (eg, visible swelling or symptoms of internal swelling). Safety and tolerability were evaluated by assessments of adverse events (AEs), laboratory analyses (clinical chemistry, hematology, and urinalysis), vital signs, electrocardiograms, and physical examinations. Investigators recorded AEs on case report forms from the time of signed consent until the last study visit. Definitions and assessment of AE severity were based on criteria listed in the 2007 Division of Microbiology and Infectious Diseases Adult Toxicity Table. The investigator or a medically qualified designee reviewed each AE to determine its relationship to the study drug in a blinded manner. At baseline, investigators discussed reporting of HAE attacks versus gastrointestinal symptoms (AEs) with all patients. Patients' QoL and treatment satisfaction were assessed via the Angioedema Quality of Life Questionnaire (AE-QoL) and the Treatment Satisfaction Questionnaire for Medication (TSQM).

The AE-QoL is a validated, angioedema-specific 17-item questionnaire. Scores range from 0 to 100, with higher scores indicating greater impairment.[16] The minimal clinically important difference (MCID) is defined as a change of 6 points.[17] The TSQM consists of 14 items. Scores range from 0 to 100, with higher scores indicating higher satisfaction.[18]

Patients were instructed to take the study drug at the same time each day with their largest meal to potentially minimize gastrointestinal side effects. Study drug compliance was based on return capsule count.

Outcomes

Baseline HAE attack rates, expressed as the number of investigator-confirmed HAE attacks per 28-day period, were calculated over the period from screening to the first dose of study drug. The HAE attacks used to calculate baseline attack rates must not have begun within 48 hours of the end of the previous attack and required treatment or caused functional impairment. The primary efficacy end point was the rate of investigator-confirmed HAE attacks during the 24-week double-blind treatment period. Investigator-confirmed attack rates were also summarized by month, defined in blocks of 28 days beginning on the first day of dosing. Secondary end points were the change from baseline in AE-QoL total scores at week 24,[16] the number and proportion of days with angioedema symptoms through 24 weeks, and the investigator-confirmed attack rates during the effective (steady-state) treatment period (day 8 to end of Part 1). Exploratory measures included the proportion of responders to the study drug, defined as at least a 50% (prespecified), 70%, or 90% (ad hoc) relative reduction in the rate of investigator-confirmed HAE attacks during treatment compared with the baseline attack rate, proportion of patients with no attacks over 24 weeks (prespecified), and rate of investigator-confirmed HAE attacks treated with on-demand medication (prespecified). The rate of on-demand medication use (ad hoc) was also calculated. Satisfaction with treatment was assessed by using TSQM scores (prespecified). Safety outcomes were assessed over the entire treatment period and included the incidence of treatment-emergent AEs (TEAEs), discontinuations due to TEAEs, serious TEAEs (TESAEs), grade 3 or 4 TEAEs, and grade 3 or 4 laboratory abnormalities.

Statistical analyses

All prespecified analyses were detailed in the statistical analysis plan. A sample size of 32 patients per group provided 94% power (2 sided; significance level 0.05) to detect a 50% attack rate reduction (target difference) between berotralstat and placebo. This sample size was calculated on the basis of results from the phase 2 study on berotralstat, assuming a placebo attack rate of 1 attack per week and a common SD of 0.55 attacks per week for placebo and berotralstat.

Primary and secondary efficacy analyses were conducted by using the intent-to-treat population. Safety analyses were done using the safety population.

All statistical summaries and analyses were performed by using SAS software, version 9.4 (SAS Institute Inc, Cary, NC). In the primary analysis, each berotralstat dose was compared with placebo by using a negative

binomial model. The number of investigator-confirmed HAE attacks was included as the dependent variable, treatment as a fixed effect, baseline investigator-confirmed attack rate as a covariate, and the logarithm of the duration on treatment as an offset variable. Analysis of the attack rate during the effective dosing period, rate of attacks treated with SOC, and rate of on-demand medication use was similarly conducted.

To account for multiplicity, the Hochberg step-up procedure was used to adjust for the comparison of active drug with placebo for 4 end points (1 primary and 3 secondary) and 2 doses (110 mg and 150 mg). The primary and secondary end points were tested in hierarchic fashion as follows: (1) rate of investigator-confirmed HAE attacks during the 24-week double-blind treatment period, (2) change from baseline in the AE-QoL (week 24 total score), (3) number and proportion of days with angioedema symptoms, and (4) rate of investigator-confirmed HAE attacks during the effective dosing period. The 2 doses were tested at the α level of 0.05 by using the Hochberg step-up procedure at each level of the hierarchy to which both doses progressed through the hierarchy. Once a dose failed, it could not be tested against further end points in the hierarchy and the remaining dose was tested at the α level of 0.025. Reported nominal *P* values are *P* values that have not been adjusted for multiplicity in hypothesis testing.

Changes in AE-QoL from baseline were assessed with a mixed model for repeated measures with fixed effects for treatment, baseline attack rate, baseline AE-QoL, visit, and a visit by treatment interaction and random effect for patient. An unstructured covariance structure was used. Statistical analysis of the proportion of days with angioedema symptoms from investigator-confirmed attacks was based on an analysis of covariance model. Analysis of TSQM scores was similar to that for AE-QoL.

To qualify for study entry, the HAE attacks occurring during the run-in period used to calculate baseline attack rates had to meet 2 additional requirements that were not required for the on-study primary end point: first, the attacks must not have begun within 48 hours of the end of the previous attack; second, they must require treatment or cause functional impairment. To allow for an appropriate comparison of the on-study attack rates with the baseline attack rate, these requirements were applied to on-study investigator-confirmed attacks to calculate an adjusted investigator-confirmed attack rate. Adjusted confirmed attack rates were used for calculating the proportion of responders. Odds ratios (ORs) were determined by using logistic regression with responder status as the outcome variable and treatment and investigator-confirmed baseline attack rate as independent variables.

Baseline characteristics were assessed for their ability to predict efficacy outcomes as an *ad hoc* analysis. For the effect of baseline characteristics on the primary analysis, negative binomial regression models were constructed with investigator-confirmed attack rate as the outcome variable and the log of treatment duration as the offset variable. A final multivariable model was obtained by using a stepwise regression process with a 20% significance level for a variable to enter the model and a 15% significance level for a variable to stay in the model. The effect of baseline characteristics on the responder status was evaluated by using logistic regression separately for responses of at least 50% and at least 70% relative reductions, by initially including 1 independent variable at a time in the univariate models.

AEs were summarized descriptively by using Medical Dictionary for Regulatory Activities–preferred terms, system organ class, and severity.

RESULTS
Study population and treatment compliance
The study investigators screened a total of 160 patients; of these patients, 121 were randomized into 1 of 3 arms: 41 patients to 110 mg, 40 patients to 150 mg, and 40 patients to placebo (1 patient was not dosed) between March 14, and October 23, 2018. The groups were generally well balanced. Patients were predominantly female, and the mean baseline HAE attack rate was approximately 3 attacks per month across treatment groups (Table I).

In all, 12 patients discontinued the study drug early (4 receiving 110 mg of berotralstat; 3 receiving 150 mg of berotralstat; and 5 receiving placebo). In addition, 5 patients discontinued the study drug because of a laboratory result abnormality or AE (3 receiving 110 mg of berotralstat; 1 receiving 150 mg of berotralstat; and 1 receiving placebo), 4 discontinued the study drug because of a perceived lack of efficacy (1 receiving 110 mg of berotralstat; 1 receiving 150 mg of berotralstat; and 2 receiving placebo), 2 withdrew consent (1 receiving 150 mg of berotralstat and 2 receiving placebo), and 1 withdrew consent for other reasons (1 receiving placebo). The last patient completed 24 weeks of treatment on April 10, 2019. The mean rate of compliance with study drug dosing was high: 98% (± an SD of 4.56), 99% (± 3.93), and 97% (± 7.53) for the 110-mg dose of berotralstat, 150-mg dose of berotralstat, and placebo groups, respectively.

End points
Berotralstat demonstrated a significant reduction in attacks at both dose levels relative to placebo: 1.65 attacks per month at 110 mg (*P* = .024). 1.31 attacks per month at 150 mg (*P* < .001). and 2.35 attacks per month with placebo (Table II). The model-based attack rate ratio comparing berotralstat with placebo was 0.7 (95% CI = 0.51-0.95) for 110 mg and 0.56 (95% CI = 0.41-0.77) for 150 mg. The reduction in mean attack rate began within the first month and was sustained throughout the 24-week period (Fig 1).

All sensitivity analyses of treatment with both doses of berotralstat were supportive of the robustness of the primary analysis outcomes.

In the 150-mg dose of berotralstat group, the rate of HAE attacks was significantly reduced in patients with 2 or more attacks at baseline (1.76 and 2.92 attacks per month for the 150-mg dose of berotralstat and placebo groups, respectively [*P* = .005]) and patients with fewer than 2 attacks per month at baseline (0.50 and 1.45 attacks per month for the 150-mg dose of berotralstat and placebo groups, respectively [*P* = .009]). In the 110-mg dose of berotralstat group, only patients in the subgroup with 2 or more attacks per month at baseline had a significant reduction in attack rate (for ≥2 attacks per month at baseline, 1.99 and 2.92 attacks per month [*P* = .035]; for <2 attacks per month at baseline, 1.06 and 1.45 attacks per month for the 110-mg dose of berotralstat and placebo groups respectively [*P* = .327]). Attacks occurring on study were categorized by anatomic location and are summarized in Table E2 (in the Online Repository at www.jacionline.org).

The secondary end point of AE-QoL total score was not significant versus placebo (least squares mean [LSM] difference from placebo: –2.77 [95% CI = –10.08 to 4.53] points in the 110-mg dose of berotralstat group [*P* = .453] and –4.90 [95% CI = –12.23 to 2.43] points in the 150-mg dose of berotralstat group [*P* = .188]) (Table II). The overall AE-QoL mean change from baseline exceeded the MCID in all treatment arms at 24 weeks (LSM difference from baseline [SE]: –12.46 [2.53] points in the 110-mg dose of berotralstat group, –14.59 [2.59] points in the 150-mg dose of berotralstat group, and –9.69 [2.64] points in the placebo group).

Although formal statistical testing was not performed on subsequent secondary end points owing to the Hochberg hierarchic rules, nominal analyses are reported for clarity. The mean

TABLE I. Baseline characteristics of the intent-to-treat population

	Berotralstat		
Characteristic	110 mg (n = 41)	150 mg (n = 40)	Placebo (n = 40)
Age at consent (y), mean (SD)	40.4 (17.5)	40.0 (14.0)	44.5 (14.1)
Female sex, no. (%)	30 (73)	23 (58)	27 (68)
Race, no. (%)*			
White	38 (93)	38 (95)	37 (93)
Weight at screening (kg), mean (SD)	78.8 (21.5)	87.6 (20.4)	84.9 (21.4)
Region, no. (%)			
North America	32 (78)	27 (68)	28 (70)
Europe	9 (22)	13 (33)	12 (30)
BMI at screening (kg/m^2), mean (SD)	27.5 (7.3)	30.4 (6.7)	29.3 (6.8)
BMI 18.5-24.9 kg/m^2 (normal weight), no. (%)	19 (46)	8 (20)	12 (30)
BMI 25-29.9 kg/m^2 (overweight), no. (%)	8 (20)	16 (40)	14 (35)
BMI ≥30 kg/m^2 (obese), no. (%)	14 (34)	16 (40)	13 (33)
Baseline investigator-confirmed attack rate, mean (SD)	2.97 (1.36)	3.06 (1.56)	2.91 (1.12)
Baseline investigator-confirmed attack rate, no. (%)			
≥2 attacks/mo	28 (68)	30 (75)	27 (68)
<2 attacks/mo	13 (32)	10 (25)	12 (30)†
Any past prophylactic treatment for HAE, no. (%)	32 (78)	30 (75)	29 (73)
Any prior androgen use, no. (%)‡	19 (46)	21 (53)	25 (63)
Any prior prophylactic C1-INH use, no. (%)§	16 (39)	21 (53)	16 (40)
Prior prophylactic treatment use within 30 days of screening, no. (%)	10 (24)	12 (30)	11 (28)

BMI, Body mass index.

The intent-to-treat population included all patients who underwent randomization.

*Race was self-reported.

†A total of 40 patients are in the analysis population; 1 patient was not dosed and therefore has no attack data for Part 1.

‡Prior androgen use was noted in the patient's HAE medical and medication history and included any of the following: androgens (unspecified), oxandrolone, methyl-testosterone, danazol, and stanozolol.

§C1-INH includes plasma-derived and recombinant C1-INH and fresh frozen plasma.

numbers of days with angioedema symptoms were 20.8 (± 19.22), 19.4 (±21.50), and 29.2 (±24.29) days for the 110-mg dose of berotralstat, 150-mg dose of berotralstat, and placebo groups, respectively. The LSM differences from placebo proportion of days with angioedema symptoms were –0.062 days (95% CI = –0.117 to –0.008; nominal *P* = .025) in the 110-mg dose of berotralstat group and –0.078 (95% CI = –0.133 to –0.023; nominal *P* = .006) in the 150-mg dose of berotralstat group. The investigator-confirmed attack rates during the effective dosing period were 1.65, 1.27, and 2.38 attacks per month in the 110-mg dose of berotralstat, 150-mg dose of berotralstat, and placebo groups, respectively.

The percentages of patients who experienced a 50% or greater reduction in adjusted investigator-confirmed attacks during the 24 weeks were 25% in the placebo group, 51% in the 110-mg dose of berotralstat group (OR = 3.042 [95% CI = 1.183-7.821]; *P* = .021), and 58% in the 150-mg dose of berotralstat group (OR = 3.913 [95% CI = 1.507-10.164]; *P* = .005 [Fig 2 and see Fig E2 in the Online Repository at www.jacionline.org]).

The 150-mg dose of berotralstat group also showed significant benefit compared with placebo in achieving at least a 70% reduction in attacks (50% vs 15%; OR = 5.63 [95% CI = 1.926-16.458]). The percentage of patients in the 150-mg dose of berotralstat group who achieved a 90% or greater reduction in attacks (23%) was not significant compared with placebo (7.5%; OR = 3.605 [95% CI = 0.886-14.663]). No difference between groups was observed in the proportion of attack-free patients.

Both the 110-mg and 150-mg dose of berotralstat groups showed a significant reduction in the rate of investigator-confirmed HAE attacks treated with SOC medication (for 110 mg, 1.29 attacks per month [nominal *P* = .015], and for 150 mg, 1.04 attacks per month [nominal *P* < .001] vs a rate of 2.05 attacks

per month for placebo). The model-based attack rate ratio was 0.63 (95% CI = 0.44-0.91) for the 110-mg dose of berotralstat group and 0.51 (95% CI = 0.35-0.75) for the 150-mg dose of berotralstat group. Additionally, the rates of SOC medication use were significantly reduced in both groups (for 110 mg of berotralstat, 1.50 doses per month [nominal *P* = .002], and for 150 mg of berotralstat, 1.29 doses per month [nominal *P* < .001] vs a rate of 2.79 doses per month for placebo). The model-based rate ratios were 0.54 (95% CI = 0.36-0.80) and 0.46 (95% CI = 0.31-0.70) for the 110-mg dose of berotralstat and 150-mg dose of berotralstat groups, respectively.

An *ad hoc* analysis was performed to assess the predictive value of baseline characteristics on the rates of investigator-confirmed attacks and responder status (≥50% or ≥70% reduction in attack rate relative to baseline) with berotralstat treatment (see Table E3 in the Online Repository at www.jacionline.org). Only treatment group was a significant predictor of response in all 3 models, specifically indicating that treatment with 150 mg of berotralstat was predictive of both a reduction in attack rate and a relative reduction of 50% or more and 70% or more in adjusted HAE attack rate.

The TSQM global satisfaction score (LSM week 24 difference from placebo of 18.9 (95% CI = 4.7-33.1; *P* = .010) and effectiveness score (LSM difference from placebo of 18.7 (95% CI = 4.0-33.4; *P* = .013) were improved relative to placebo for the 150-mg dose of berotralstat treatment group at 24 weeks (see Fig E3 in the Online Repository at www.jacionline.org). The scores for side effects and convenience for patients taking berotralstat were not differentiated from those for patients taking placebo, although it is noteworthy that convenience scores improved in comparison with baseline for all 3 treatment groups.

TABLE II. Summary of end points and additional analyses, intent-to-treat population

|	Berotralstat		
End point	110 mg (n = 41)	150 mg (n = 40)	Placebo (n = 40)
Primary			
Estimated monthly investigator-confirmed attack rate through week 24*	1.65	1.31	2.35
Attack rate ratio relative to placebo (95% CI)	0.70 (0.51-0.95)	0.56 (0.41-0.77)	—
P value	.024	<.001	—
Secondary			
CFB to week 24 in AE-QoL total score, LSM (SE)†	−12.46 (2.53)	−14.59 (2.59)	−9.69 (2.64)
Difference from placebo, LSM (95% CI)	−2.77 (−10.08 to 4.53)	−4.90 (−12.23 to 2.43)	—
P value	.453	.188	—
Proportion of days with angioedema symptoms, LSM (SE)‡	0.134 (0.019)	0.119 (0.019)	0.197 (0.020)
Difference from placebo, LSM (95% CI)	−0.062 (−0.117 to −0.008)	−0.078 (−0.133 to −0.023)	—
Nominal P value	.025	.006	—
Estimated monthly confirmed attack rate over the effective dosing period (day 8 to week 24)*§	1.65	1.27	2.38
Attack rate ratio relative to placebo (95% CI)	0.70 (0.51-0.96)	0.54 (0.39-0.74)	—
Nominal P value	.026	<.001	—

CFB, Change from baseline.

*Investigator-confirmed attack rate is defined as the total number of investigator-confirmed HAE attacks experienced in the entire Part 1 dosing period. Statistical analysis is based on a negative binomial regression model in which the number of investigator-confirmed attacks is included as the dependent variable, the treatment is included as a fixed effect, the baseline investigator-confirmed attack rate is included as a covariate, and the logarithm of duration of treatment is included as an offset variable.

†The AE-QoL scores range from 0 (best) to 100 (worst). Statistical analysis is based on a mixed-model repeated measures analysis with baseline investigator-confirmed attack rate, baseline AE-QoL, treatment, visit, and visit-by-treatment interaction included as fixed effects. Patient is included as a random effect.

‡The proportion of days with angioedema symptoms due to investigator-confirmed attacks is based on the number of days with reported symptoms from investigator-confirmed attacks in Part 1 and the number of days the patient was receiving treatment in Part 1. Statistical analysis is based on an analysis of covariance model with baseline investigator-confirmed attack rate as a covariate and treatment included as a fixed effect.

§The effective dosing period is the steady-state dosing period defined as days 8 to 168.
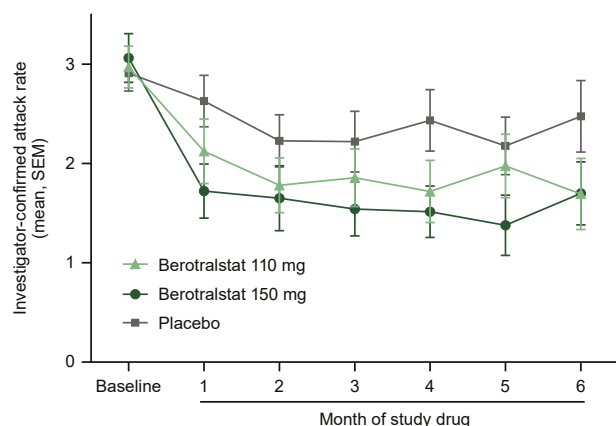
FIG 1. Mean investigator-confirmed attack rate by month in the intent-to-treat population.

Safety and tolerability

The percentage of patients experiencing at least 1 TEAE was similar in all 3 arms over the 24-week period (83% with the 110-mg dose of berotralstat, [n = 34]; 85% with the 150-mg dose of berotralstat, [n = 34]; and 77% with placebo, [n = 30]) (Table III).

The TEAEs that occurred most commonly (≥10% in any treatment group) and more frequently with berotralstat (>2-patient difference in either arm compared in the placebo arm) on study were abdominal pain, vomiting, diarrhea, and back pain. Gastrointestinal abdominal TEAEs were generally grade 1 or 2 (see Table E4 in the Online Repository at www.jacionline.org) and self-limited. Events of vomiting, diarrhea, or abdominal pain had a median duration of 2 days in the 150-mg of berotralstat arm (95% CI = 1.0-7.0) versus 1 day in the placebo arm (95% CI = 0.0-7.0). Gastrointestinal abdominal TEAEs occurred

primarily within the first month of treatment (see Fig E4 in the Online Repository at www.jacionline.org).

There was 1 TESAE in the 110-mg of berotralstat arm (plasma cell myeloma) and 3 TESAEs in the placebo arm (uterine leiomyoma, pneumonia, and 1 patient with diverticular hemorrhage and a transient ischemic attack). All TESAEs were reported by the investigators as unrelated to the study drug. Three patients experienced drug-related grade 3 or 4 AEs, all in the 110-mg of berotralstat arm (2 patients with abdominal pain and 1 patient with purpura).

Five patients discontinued treatment because of TEAEs: 3 patients in the 110-mg of berotralstat arm (1 because of dyspepsia, nausea, and vomiting; 1 because of plasma cell myeloma; and 1 because of purpura); 1 patient in the 150-mg of berotralstat arm who experienced an asymptomatic transaminase level increase; and 1 patient in the placebo arm who experienced depression. All TEAEs leading to discontinuation were assessed as possibly or probably related to the study drug, except the event of plasma cell myeloma (unrelated), which had laboratory evidence at baseline.

Grade 3 and 4 laboratory abnormalities occurred in all 3 treatment arms. Only 1 grade 3 or 4 laboratory result abnormality was reported by the investigator as an AE during berotralstat therapy: an asymptomatic grade 4 elevation in alanine aminotransferase level (in the 150-mg dose of berotralstat arm, reported as a grade 1 AE, possibly related) in a patient with previous androgen exposure who discontinued the study drug.

DISCUSSION

This study demonstrated that berotralstat, an oral, once-daily plasma kallikrein inhibitor, significantly reduced the rate of investigator-confirmed HAE attacks compared with placebo at
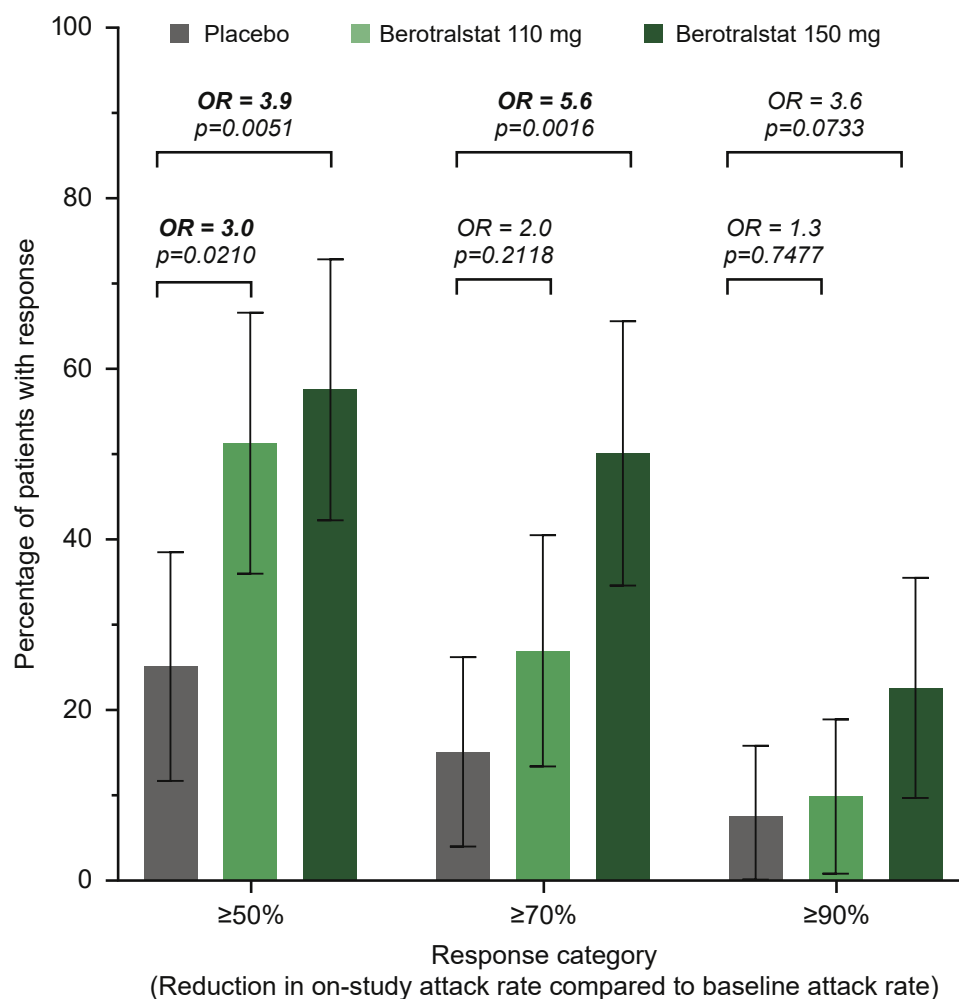
FIG 2. Percentage of patients with a 50% or greater, 70% or greater, or 90% or greater relative reduction from baseline (95% CI) in adjusted investigator-confirmed attack rate in the intent-to-treat population (exploratory). ORs in bold text indicate statistical significance. An adjusted confirmed HAE attack rate was computed for the determination of the responder end points by comparing postbaseline attack rates with baseline attack rates. For adjusted confirmed HAE attack rates, attacks during the treatment period must not have begun within 48 hours of the end of a previous attack and must have been treated, have required medical attention, or have been documented to cause functional impairment, matching the rules for the baseline attack rates.

both studied doses (110 mg per day and 150 mg per day) over the 24-week study period. The reduction in mean attack rate began within the first month of treatment and was sustained throughout the entire 24-week period.

The AE-QoL scores meaningfully improved in all study arms beyond the AE-QoL MCID of 6. Other secondary and exploratory end points were consistent with the primary end point. There were reductions in days with angioedema symptoms, with approximately 10 and 13 more symptom-free days over 24 weeks for the 110-mg and 150-mg dose of berotralstat groups, respectively, as well as improvements in attack rate within the effective dosing period. The TSQM showed clear superiority for berotralstat treatment versus placebo in the global and effectiveness domains. Exploratory and *ad hoc* analyses demonstrated greater reductions in the rate of investigator attacks treated with SOC medication (with 110 mg of berotralstat, −36.9%; with 150 mg of berotralstat, −49.2%) and SOC medication use (with 110 mg of berotralstat,

−46.3%; with 150 mg of berotralstat, −53.6%) than would be expected based solely on the reduction in attack rates at both doses (with 110 mg of berotralstat, −30.0 %; with 150 mg of berotralstat, −44.2%), which may suggest reduced attack severity in patients receiving berotralstat.

Berotralstat was safe and generally well tolerated at both doses. The most frequently reported AEs with berotralstat were gastrointestinal symptoms (gastrointestinal abdominal TEAEs occurred in 17 patients [42%] receiving 110 mg of berotralstat; 20 patients [50%] receiving 150 mg of berotralstat; and 14 patients [36%] receiving placebo). These symptoms were generally mild and transient, with a pattern of remitting with continued treatment. Investigators did not observe any related TESAEs, and AE discontinuation rates were similar for patients in the 150-mg dose of berotralstat and placebo groups.

The patients in this study reflected the wide distribution of disease severity across the overall population of patients with

TABLE III. Summary of TEAEs, safety population

TEAE, no. (%)	Berotralstat		Placebo (n = 39)
	110 mg (n = 41)	150 mg (n = 40)	
Any TEAE	34 (83)	34 (85)	30 (77)
Any TESAE	1 (2)	0	3 (8)
Any drug-related TESAE	0	0	0
Any grade 3 or 4 TEAE*	5 (12)	1 (3)	4 (10)
TEAEs leading to discontinuation	3 (7)	1 (3)	1 (3)
TEAE (EOSI), investigator-identified rash	0	1 (3)	0
Drug-related investigator-identified rash	0	0	0
Most frequent TEAEs (≥10% in any treatment arm)			
Upper respiratory tract infection	13 (32)	12 (30)	11 (28)
Nausea	6 (15)	6 (15)	7 (18)
Abdominal pain	4 (10)	9 (23)	4 (10)
Vomiting	4 (10)	6 (15)	1 (3)
Diarrhea	4 (10)	6 (15)	0
Headache	3 (7)	4 (10)	2 (5)
Back pain	1 (2)	4 (10)	1 (3)

EOSI, Event of special interest.

AEs were coded by using the Medical Dictionary for Regulatory Activities MedDRA, version 19.1. Only TEAEs, defined as those events that occurred after initiation of study drug through the initiation of dosing in Part 2 or through the last dose of study drug in Part 1 plus 30 days for patients who did not continue into Part 2, were summarized.

The terms *upper respiratory tract infection*, *abdominal pain*, and *diarrhea* are medical concepts that include multiple preferred terms. The term *upper respiratory tract infection* includes the preferred terms *nasopharyngitis*, *upper respiratory tract infection*, and *viral upper respiratory tract infection*. The term *abdominal pain* includes the preferred terms *abdominal pain*, *abdominal discomfort*, *abdominal pain upper*, and *abdominal tenderness*. The term *diarrhea* includes the preferred terms d*iarrhea* and *frequent bowel movement*.

*Grades 3 and 4 represent severe or life-threatening AEs, respectively.

HAE. The mean attack rate during the prospective run-in period (approximately 3 attacks per month) was greater than that reported in the overall population of those with HAE (2 attacks per month)[6] but was well balanced between treatment arms.

Patients assigned to receive berotralstat, 150 mg, had approximately 6-fold higher odds of having a 70% reduction in attack rate relative to baseline than did those patients assigned to placebo. No clear baseline discriminator was found that could be used to preidentify patients with a robust response. In a multivariable regression analysis, only study treatment assignment (with 150 mg of berotralstat) was consistently a strong predictor of response. Accordingly, the percentages of patients who experienced a 50% or greater or 70% or greater response to 150 mg of berotralstat were similar, regardless of whether the baseline attack rate was at least 2 attacks or fewer than 2 attacks per month. Age was a significant predictor of a 70% or greater relative reduction (younger age was associated with a greater chance of at least a 70% relative reduction); however, age was not a significant predictor of either at least a 50% relative reduction or on-treatment attack rate. This analysis suggests that berotralstat could become a viable treatment option for any patient with HAE, regardless of baseline characteristics.

A limitation of this study was the relatively short treatment period of 24 weeks for evaluating prophylactic therapy in a lifelong disorder. The study is ongoing, and future analyses will provide longer-term results. Future analyses of treatment response will include assessment of pharmacokinetic and pharmacodynamic parameters. Additional long-term data will be generated by an ongoing open-label safety study of berotralstat (APeX-S; NCT03472040). Additionally, the number of patients in each treatment group was relatively small. The results from this trial do not provide guidance for the selection of patients with HAE–C1-INH who would most likely respond to berotralstat. At 150 mg per day, most patients benefited from treatment, and 50% of patients achieved at least a 70% reduction of attack rate.

Berotralstat specifically targets plasma kallikrein and is the first oral plasma kallikrein inhibitor that has been shown to reduce HAE attack rates in a phase 3 trial. Berotralstat has several important advantages compared with other plasma kallikrein–targeted treatments. It does not require preparation or refrigeration and could be a simple, once-daily oral treatment for patients. Patients' preference for oral medications over injectables is well documented,[19,20] and many patients with HAE–C1-INH strongly desire an effective oral prophylactic medication. Long-term continued use of an effective and tolerable oral prophylactic medication may have important impacts on patient QoL.

In conclusion, the APeX-2 study demonstrated that berotralstat is an effective oral prophylactic treatment for patients with HAE–C1-INH. The most favorable benefit-to-risk profile was observed at a dose of 150 mg per day. The combination of efficacy, safety, and tolerability with convenient oral, once-daily dosing will make berotralstat an important addition to the HAE–C1-INH therapeutic armamentarium. A key goal in treating HAE–C1-INH is to allow patients to live a normal life. Providing them with an effective, oral, targeted prophylactic medication is a major step toward that goal.

Clinical implication: Evidence from this study suggests that berotralstat may provide an important oral alternative to injectable prophylactic options for patients with HAE.

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METHODS
Main inclusion criteria

The main inclusion criteria were as follows:

1. Males and nonpregnant, nonlactating females aged 18 years or older (main study) or aged 12 to 17 years (substudy).
2. Able to provide written, informed consent. Patients aged 12 to 17 years who are being screened for the substudy must be able to read, understand, and be willing to sign an assent form in addition to having a caregiver providing informed consent.
3. Patient weight of 40 kg or more.
4. A clinical diagnosis of hereditary angioedema type 1 or 2, defined as having a C1-INH functional level less than 50% and a complement 4 (C4) level less than the LLN reference range, as assessed during the screening period.
   - In the absence of a low C4 value drawn during the intercritical period (ie, the patient is not having an HAE attack), 1 of the following is acceptable to confirm the diagnosis of HAE: (1) a *SERPING1* gene mutation known or likely to be associated with HAE type 1 or 2 assessed during the screening period; (2) a confirmed family history of C1-INH deficiency; or (3) C4 redrawn and retested during an attack in the screening period with the results below the LLN reference range.
   - For patients with C1-INH function of at least 50% but less than the assay LLN, a *SERPING1* gene mutation known or likely to be associated with HAE type 1 or 2, as assessed during the screening period, *or* a repeat C1-INH functional level less than 50% will be considered acceptable for enrollment.
5. Access to and ability to use 1 or more acute medications approved by the relevant competent authority for the treatment of acute HAE attacks (icatibant, plasma-derived C1-INH, ecallantide, or recombinant C1-INH). Cinryze (C1-INH [human]; Takeda Pharmaceutical Company Limited, Lexington, Mass) used for acute treatment of HAE attacks is an acceptable medication for this purpose.
6. Patients must be medically appropriate for on-demand treatment as the sole medicinal management for their HAE during the study.
7. The patient must have had 2 or more HAE attacks that meet all of the following requirements during the run-in period (a maximum of 56 days from the screening visit):
   - The attacks are unique. A unique attack is defined as an attack that does not begin within 48 hours of the end of a previous attack.
   - The attacks must have either been treated, required medical attention, or been documented to cause functional impairment based on the patient's entry in the diary. Functional impairment is defined as the patient not being able to perform his or her daily activities without restriction (ie, the patient records that he or she is at least slightly restricted in his or her daily activities during the HAE attack).
   - The attacks must include symptoms of swelling. Symptoms of swelling, in addition to visible swelling, may also include symptoms in the oropharyngeal or abdominal regions, which are indicative of internal swelling.
   - The attacks are otherwise confirmed by the investigator to be HAE attacks.
   - Patients who have recorded 2 such attacks may be randomized to receive study drug beginning on or after day 28 of the run-in period; patients who have recorded 3 or more such attacks may be randomized beginning on or after day 14 of the run-in period. Under no circumstances should the run-in attack requirement for eligibility be disclosed to the study patients.
8. Female and male patients must agree to the contraception requirements and must meet the inclusion criteria regarding contraception and contraception of female partners (as applicable).
9. In the opinion of the investigator, the patient is expected to adequately comply with all required study procedures for the duration of the study. The patient must demonstrate adequate compliance with all study procedures required from the screening visit through randomization, including diary recording of HAE attacks beginning at the screening visit.
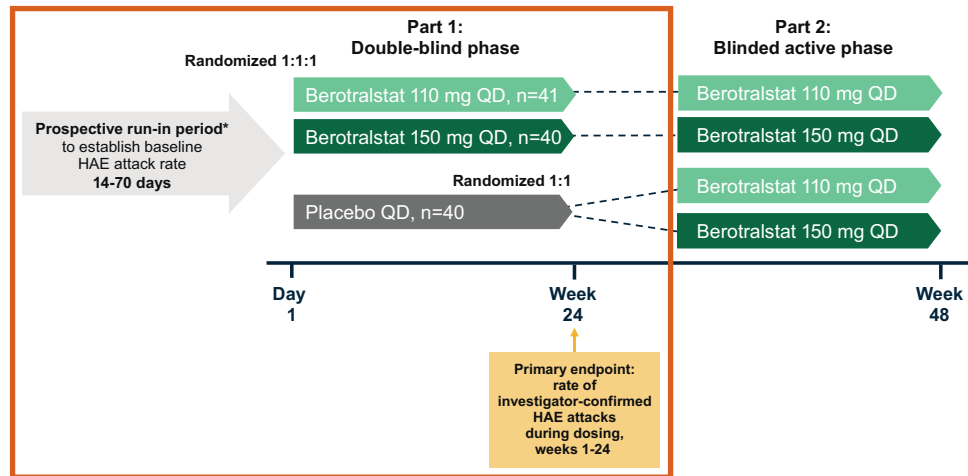
FIG E1. Study diagram. *Patients must have had 2 or more discrete attacks in the first 56 days of the run-in period to be eligible for study. *QD,* Once daily.
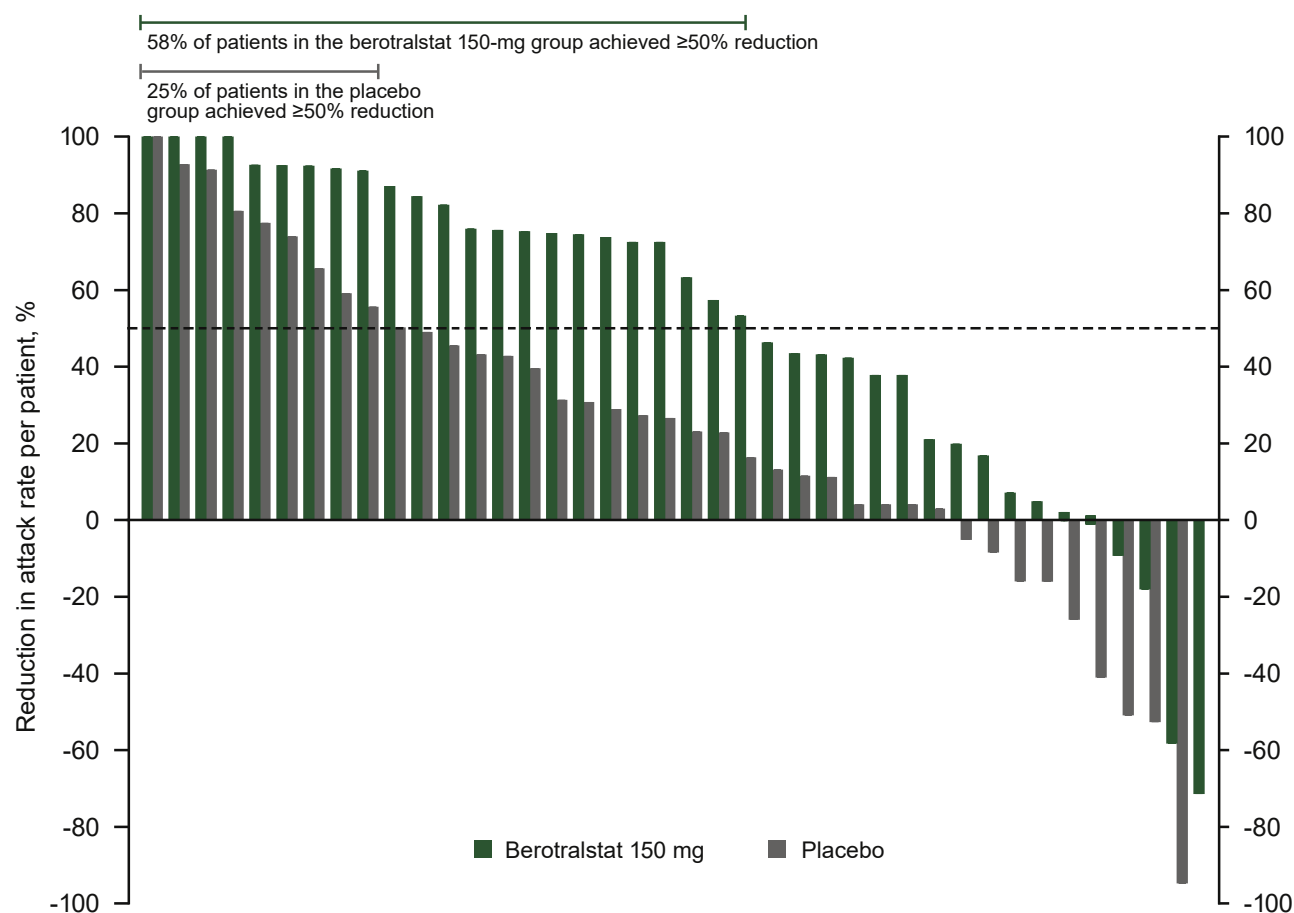
FIG E2. Waterfall plot of relative reduction from baseline in adjusted investigator-confirmed attack rate for 150 mg (*green*) and placebo (*gray*) for the intent-to-treat population.
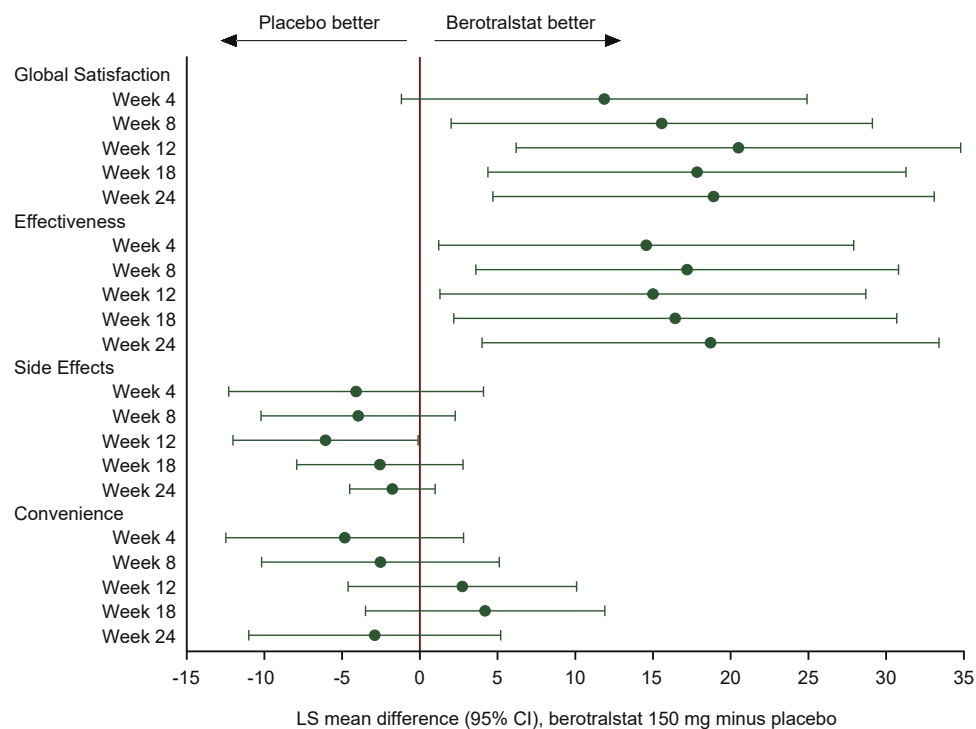
FIG E3. Forest plot of difference from placebo (95% CI) for TSQM scores, 150-mg dose of berotralstat (intent-to-treat population; *ad hoc* analysis). TSQM scores ranged from 0 to 100, with higher scores indicating higher satisfaction. *LS,* Least squares.

Patients on study drug						
Berotralstat 110 mg	41	41	41	41	40	38
Berotralstat 150 mg	40	40	37	37	37	37
Placebo	39	39	38	37	36	34
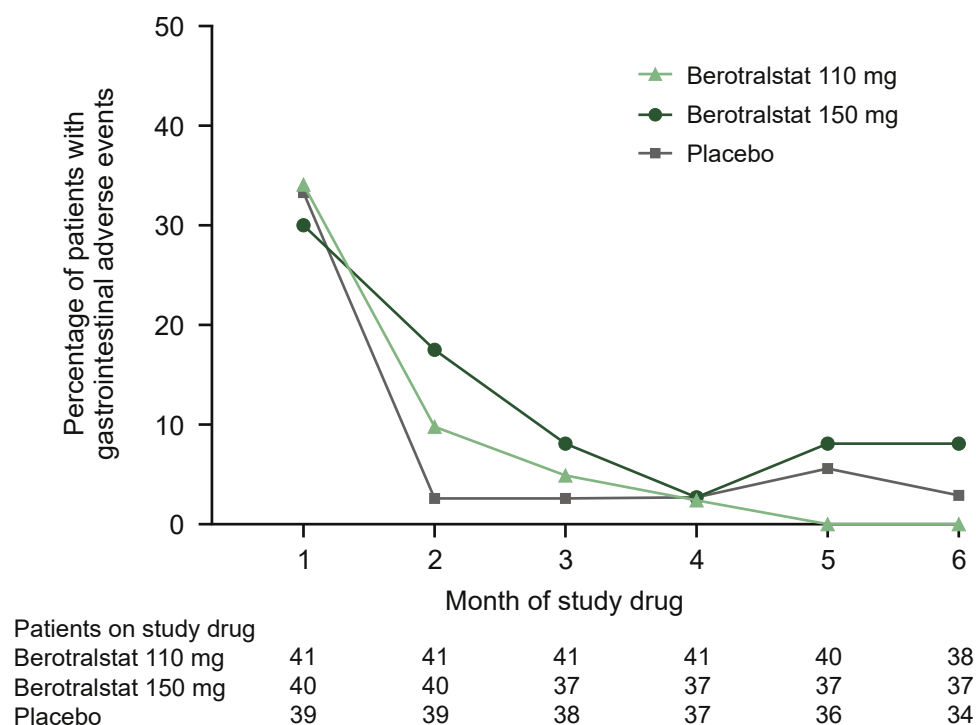
FIG E4. Plot of treatment-emergent gastrointestinal (GI) abdominal-related TEAEs by study month (safety population; *ad hoc* analysis). GI abdominal-related TEAEs were defined as TEAEs with a preferred term within the MedDRA Medical Dictionary for Regulatory Activities, version 19.1, hierarchy under the high-level group terms of (1) GI signs and symptoms or (2) GI motility and defecation conditions. Study month was defined in 28-day intervals (eg, month 1 includes days 1-28, month 2 includes days 29-56). AEs were categorized into months based on start day. Patients were considered as having an event in the month if the day of onset was included in the 28-day interval; patients could be counted each month if they had a new GI TEAE. "Patients on study drug" include those who were receiving a study drug in the month of interest or had discontinued drug within 30 days of the start of the period.

TABLE E1. List of study investigators and locations

Investigator name	Location
United States	
Anderson, John	Birmingham, Ala
Banerji, Aleena	Boston, Mass
Bernstein, Jonathan	Cincinnati, Ohio
Busse, Paula	New York, NY
Craig, Timothy	Hershey, Pa
Diaz, Joseph	San Antonio, Tex
Fritz, Stephen	Clackamas, Ore
Gower, Richard	Spokane, Wash
Jacobs, Joshua	Walnut Creek, Calif
Johnston, Douglas	Charlotte, NC
Li, H. Henry	Chevy Chase, Md
Lumry, William	Dallas, Tex
McNeil, Donald	Columbus, Ohio
Mumneh, Nayla	Piscataway, NJ
Otto, William	Austin, Tex
Riedl, Marc	San Diego, Calif
Shapiro, Ralph	Plymouth, Minn
Sitz, Karl	Little Rock, Ark
Soteres, Daniel	Colorado Springs, Colo
Tachdjian, Raffi	Santa Monica, Calif
Wedner, H. James	St. Louis, Mo
Canada	
Gagnon, Remi	Quebec, Quebec
Sussman, Gordon	Toronto, Ontario
Yang, William	Ottawa, Ontario
Europe	
Aygören-Pürsün, Emel	Frankfurt, Germany
Bara, Noemi	Jud Mures, Romania
Bethune, Claire	Plymouth, United Kingdom
Bouillet, Laurence	Grenoble, France
Caballero, Teresa	Madrid, Spain
Fain, Oliver	Antoine, Paris
Farkas, Henriette	Budapest, Hungary
Grivcheva-Panovska, Vesna	Skopje, Macedonia
Hakl, Roman	Brno, Czech Republic
Hanzlíková, Jana	Plzen, Czech Republic
Kiani, Sorena	London, United Kingdom
Kinaciyan, Tamar	Vienna, Austria
Manson, Ania	Cambridge, United Kingdom
Maurer, Marcus	Berlin, Germany
Tejerina, Teresa Gonzalez-Quevedo	Madrid, Spain
Yong, Patrick	Camberley, United Kingdom

TABLE E2. Summary of investigator-confirmed attack rates by anatomic location category (entire dosing period), intent-to-treat population

| | Berotralstat | | | | Placebo (n = 40) |
| | 110 mg (n = 41) | | 150 mg (n = 40) | | |
Location attack rate	Rate per 28 days*	Active vs placebo, % (95% CI)	Rate per 28 days*	Active vs placebo, % (95% CI)	Rate per 28 days*
Abdominal-only investigator-confirmed attack rate†‡	0.30	−22% (−66 to 76)	0.17	−56% (−81 to 5)	0.39
Peripheral-only investigator-confirmed attack rate†§	0.84	−32% (−56 to 7)	0.51	−58% (−74 to −33)	1.23
Mixed-location investigator-confirmed attack rate†‖	0.50	−31% (−57 to 10)	0.62	−15% (−47 to 37)	0.72
Laryngeal investigator-confirmed attack rate†¶#	0.07	−59% (−83 to −2)	0.06	−63% (−85 to −10)	0.17

The attack location was derived on the basis of the specific symptoms reported in the electronic diary for an investigator-confirmed attack.

*Statistical analysis was based on a negative binomial regression model. The number of investigator-confirmed attacks was included as the dependent variable, the treatment was included as a fixed effect, the baseline investigator-confirmed attack rate was included as a covariate, and the logarithm of duration on treatment was included as an offset variable.

†Investigator-confirmed attack rate was defined as the total number of investigator-confirmed HAE attacks experienced in the entire Part 1 dosing period.

‡Abdominal-only symptoms include nausea, vomiting, abdominal discomfort, cramps (colicky pain), and diarrhea.

§Peripheral-only symptoms include visible swelling of the face/head, neck (outer swelling), legs, buttocks/genitals, eyes, arms, feet, stomach (outside), mouth/tongue/lips, hands, chest/back, and joints, as well as internal swelling or symptoms of internal swelling in the airways, such as lump in throat/tightness, change in voice, difficulty swallowing, difficulty breathing, headache, fatigue, and pink rings (erythema marginatum).

‖The mixed location was used when an attack had at least 1 symptom from the abdominal-only and peripheral-only symptom lists. Diarrhea, erythema marginatum, headache, and/or fatigue could not be selected alone or in combination without another symptom(s) of swelling or internal swelling having been considered an investigator-confirmed attack.

¶Laryngeal attacks were attacks with visible swelling in the mouth/tongue/lips or any of the following internal swelling symptoms: lump in throat (tightness), difficulty swallowing, change in voice, or difficulty breathing.

#*Ad hoc* analysis.

TABLE E3. Relationship of baseline characteristics and the rate of investigator-confirmed attacks, a 50% or greater relative reduction, and a 70% or greater relative reduction: Final multivariable model

Variable	Rate of investigator-confirmed attacks*		≥50% Responder status†		≥70% Responder status†	
	P value	Significant predictor‡	*P* value	Significant predictor‡	*P* value	Significant predictor‡
Treatment group (150 mg vs placebo)	**<.001**	**Yes**§	**.006**	**Yes**§	**.005**	**Yes**§
Treatment group (110 mg vs placebo)	**.018**	**Yes**§	**.028**	**Yes**§	.311	No§
Baseline attack rate	**<.001**	**Yes**	—	—	.088	No
Prior prophylactic use within 30 days of screening	—	—	.079	No	.073	No
Sex	.146	No	—	—	—	—
Geographic region	.101	No	—	—	—	—
Prior androgen use	—	—	.146	No	—	—
Age	—	—	—	—	**.031**	**Yes**
Weight	—	—	—	—	.120	No

BMI, Body mass index.

Baseline characteristics include treatment group, age, prior androgen use (yes/no), prior prophylactic medicine within 30 days of screening (yes/no), baseline attack rate, categorized baseline attack rate (<2 or ≥2 attacks per month), geographic region (Europe vs North America), race (white or other), weight, weight group (≥ or < median weight of 78.96 kg), BMI, BMI group (normal weight, overweight, obese), sex (male/female), C1-INH functional level at screening, and C4 level at screening (< LLN vs ≥ LLN). Bold text indicates a statistically significant correlation. Em dash indicates that the variable was not included in the initial run of the multivariable model based on the stepwise selection process; therefore, *P* values were not calculated. Bold text indicates a statistically significant correlation. Em dash indicates that the variable was not included in the initial run of the multivariable model based on the stepwise selection process; therefore, *P* values were not calculated.

*Based on examination of the Akaike information criteria from the corresponding univariate models; continuous weight and baseline attack rate were used over their categoric version.

†Based on examination of the univariate models; baseline attack rate and weight category variables were used over their continuous analogs.

‡*P* value of .05 or less signifies a predictor of attack rate, a 50% or greater relative reduction, or a 70% or greater relative reduction.

§Treatment group is a single variable in the model with values of 110 mg, 150 mg, or placebo. Comparisons between individual groups (ie, 110 mg vs placebo and 150 mg vs placebo) are presented.

TABLE E4. Summary of GI abdominal-related TEAEs

TEAE type, no. (%)	Berotralstat		Placebo (n = 39)
	110 mg (n = 41)	150 mg (n = 40)	
Any GI abdominal TEAE*	17 (42)	20 (50)	14 (36)
Any drug-related GI abdominal TEAE†	14 (34)	14 (35)	11 (28)
Any drug-related GI abdominal TESAE	0	0	0
Any grade 3 or 4 GI abdominal TEAE	2 (5)	0	0
GI abdominal TEAEs leading to discontinuation of study drug	1 (2)	0	0
Any GI abdominal TEAE that required use of concomitant medication	5 (12)	4 (10)	3 (8)

GI, Gastrointestinal.

TEAEs are defined as those events that occur after initiation of study drug through the initiation of dosing in Part 2 or through the last dose of study drug in Part 1 plus 30 days for patients who do not continue into Part 2.

*GI abdominal-related AE is any AE with a preferred term within the Medical Dictionary for Regulatory Activities, version 19.1, hierarchy under the high-level group terms of (1) GI signs and symptoms or (2) GI motility and defecation conditions.

†A drug-related GI abdominal TEAE is defined as any GI abdominal TEAE for which the investigator defines the relationship as possibly related, probably related, or definitely related.