Gastroenterology 2017;152:787–798

No Difference Between Latiglutenase and Placebo in Reducing Villous Atrophy or Improving Symptoms in Patients With Symptomatic Celiac Disease

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BACKGROUND & AIMS: Gluten ingestion leads to symptoms and small intestinal mucosal injury in patients with celiac disease. The only option is the strict lifelong exclusion of dietary gluten, which is difficult to accomplish. Many patients following a gluten-free diet continue to have symptoms and have small intestinal mucosal injury. Nondietary therapies are needed. We performed a phase 2 study of the ability of latiglutenase, an orally administered mixture of 2 recombinant gluten-targeting proteases, to reduce mucosal morphometric measures in biopsy specimens from patients with celiac disease. **METHODS:** We performed a double-blind, placebo-controlled, dose-ranging study to assess the efficacy and safety of latiglutenase in 494 patients with celiac disease (with moderate or severe symptoms) in North America and Europe, from August 2013 until December 2014. Participants reported following a gluten-free diet for at least 1 year before the study began. Patients with documented moderate or severe symptoms and villous atrophy (villous height:crypt depth ratio of $\leq$2.0) were assigned randomly to groups given placebo or 100, 300, 450, 600, or 900 mg latiglutenase daily for 12 or 24 weeks. Subjects completed the Celiac Disease Symptom Diary each day for 28 days and underwent an upper gastrointestinal endoscopy with duodenal biopsy of the distal duodenum at baseline and at weeks 12 and 24. The primary end point was a change in the villous height:crypt depth ratio. Secondary end points included numbers of intraepithelial lymphocytes, serology test results (for levels of antibodies against tissue transglutaminase-2 and deamidated gliadin peptide), symptom frequencies, and safety. **RESULTS:** In a modified intent-to-treat population, there were no differences between latiglutenase and placebo groups in change from baseline in villous height:crypt depth ratio, numbers of intraepithelial lymphocytes, or serologic markers of celiac disease. All groups had significant improvements in histologic and symptom scores. **CONCLUSIONS:** In a phase 2 study of patients with symptomatic celiac disease and histologic evidence of significant duodenal mucosal injury, latiglutenase did not improve histologic and symptom scores when compared with placebo. There were no significant differences in change from baseline between groups. ClinicalTrials.gov no: NCT01917630.

Keywords: Pathology; Healing; Inflammation; Treatment.

Celiac disease is an acquired chronic immune disorder occurring in individuals genetically susceptible to dietary gluten. It affects 1%–2% of the population[1–7] and is characterized by an inflammatory reaction that is accompanied by atrophy of the mucosal villi and hypertrophy of crypts.[8] Lifelong avoidance of dietary gluten is currently the only treatment option.[9] The daily intake of gluten is approximately 15–20 g in the typical Western diet.[10–12] Celiac disease has a wide range of clinical manifestations that can include acute gastrointestinal (GI) disturbances, chronic GI symptoms, malabsorption, or weight loss.

For patients with celiac disease, lifelong gluten exclusion needs to be followed strictly to reduce the risk of complications, including bone disorders, infertility, cancer, and an increase in overall mortality.[5,9,13–15] However, following a completely gluten-free diet (GFD) is challenging; even highly motivated patients are affected by inadvertent or background exposure to gluten, resulting in ongoing damage in the small intestine.[16,17] Such persistent injury can result in excess morbidity and/or mortality.[17,18] Thus, there is a need for nondietary therapies to be developed for celiac disease.[19]

The glutenase latiglutenase (formerly ALV003), is an orally administered, fixed-dose (1:1 ratio by weight) mixture of 2 gluten-targeting proteases (ALV001 and ALV002): ALV001 is a modified recombinant version of cysteine endoprotease B, isoform 2 from barley (*Hordeum vulgare*), and ALV002 is a modified recombinant version of a prolyl endopeptidase from the bacterium *Sphingomonas capsulata*. Gluten has a high proline and glutamine content, which makes it resistant to proteolysis by gastric,

Abbreviations used in this paper: **AE, adverse events; ANCOVA, analysis of covariance; CDSD, celiac disease symptom diary; DGP, deamidated gliadin peptide; GFD, gluten-free diet; GI, gastrointestinal; ICDSQ, Impact of Celiac Disease Symptoms Questionnaire; IEL, intraepithelial lymphocyte; MITT, Modified Intent-to-Treat; PGI-S, Patient Global Impression-Symptoms; PRO, patient-reported outcome; SF-12, Short-Form 12; TG2, tissue transglutaminase-2; Vh:Cd, villous height to crypt depth ratio.**

Most current article

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2016.11.004

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pancreatic, and intestinal brush-border endoproteases and exoproteases, which have poor specificity for peptide bonds adjacent to proline and glutamine residues.[20–22] The 2 gluten-specific proteases that comprise latiglutenase show complementary substrate sequence and chain length specificity that proteolyze gluten at specific glutamine and proline residues, respectively, reducing the immunogenic potential of those gluten-derived peptides that activate T cells derived from patients with celiac disease.[23–26]

Latiglutenase previously has been shown to attenuate mucosal injury induced by a daily 2-g gluten challenge for 6 weeks in a prior randomized, placebo-controlled trial.[27] In contrast, this new study targeted symptomatic patients who had been on a GFD for at least 1 year. Subjects reported at least 1 moderate or severe symptom related to possible gluten exposure during the 28-day period before screening and had evidence of ongoing gluten-sensitive enteropathy before randomization. The objectives of this study were to determine the effect of different dose levels of latiglutenase administered for 12 weeks on mucosal morphometry as measured by the villous height:crypt depth ratio (Vh:Cd), intraepithelial lymphocyte (IEL) density, celiac serologies, celiac disease–associated symptom frequencies, and safety.

Patients and Methods

Study Design

This was a multicenter, randomized, double-blind, placebo-controlled, dose-ranging study in symptomatic established patients with celiac disease following a GFD for at least 1 year before randomization, and was conducted in the United States, Canada, Finland, Norway, Ireland, and the United Kingdom.

Screening and Enrollment

Adult outpatients (age, 18–80 y) were required to have physician-diagnosed celiac disease, following a GFD for 1 year or more, and self-report at least 1 gastrointestinal symptom (eg, diarrhea, constipation, abdominal pain, bloating, or nausea) as moderate or severe during the 28 days before screening were recruited to participate in the study.

A schematic summary of the study is shown in Figure 1. Patients who met protocol enrollment criteria began study period 1 and were instructed to complete the celiac disease–specific patient-reported outcome (PRO) instrument called the Celiac Disease Symptom Diary (CDSD) by telephonic interactive voice response system daily for 28 days. Patients who had at least 1 moderate or severe symptom (as defined in Supplementary Table 1) contained in the CDSD during days 15–28 underwent an upper GI endoscopy with duodenal biopsy of the distal duodenum. Mucosal biopsy specimens were processed centrally and the Vh:Cd was assessed by an expert gastrointestinal pathologist.

Patients who met all randomization criteria and had a Vh:Cd of 2.0 or less were randomized to 1 of 6 treatment groups (placebo, or 100, 300, 450, 600, or 900 mg latiglutenase in a ratio of 3:1:2:1:2:1, respectively) and entered study period 2. The randomization was stratified by screening celiac serologic status (positive was defined as greater than the normal range for any of the 3 serology tests, tissue transglutaminase-2 IgA [TG2-IgA], deamidated gliadin peptide-IgA [DGP-IgA], or deamidated gliadin peptide-IgG [DGP-IgG]). Patients who failed to meet the CDSD symptom or the Vh:Cd criteria during study period 1 were withdrawn from the study. The target accrual for the study was approximately 500 randomized patients. The complete entry criteria are listed in Supplementary Table 2.

Once randomized, patients who entered study period 2 were trained to self-administer study medication (latiglutenase or placebo) up to 3 times per day with each gluten-free major meal (eg, breakfast, lunch, and dinner) for 12 weeks and complete the CDSD daily. Patients who discontinued participation in the study before week 6 of study period 2 were not required to have an end-of-treatment duodenal biopsy performed.

Approximately 120 patients in North America who completed the 12-week, double-blind treatment and underwent the week 12 biopsy were invited to receive their originally assigned study treatment for an additional 12 weeks (study period 3).

A safety follow-up visit occurred 4 weeks after the end of the treatment period (week 16 or week 28).

Dose Administration and Treatment Compliance

Patients were instructed to take the study medication with each gluten-free major meal (breakfast, lunch, and dinner); if a meal was skipped, then the patient did not take the study medication. Patients completed a study medication diary, recording each dose and meal with verification of compliance at each visit and at end of study (see the Supplementary Materials and Methods section: Verification of Compliance). Latiglutenase and placebo were provided to patients in the form of free-flowing powders supplied in separate foil stick-packs and a separate sachet that contained all flavoring, buffers, and excipients. The latiglutenase and placebo drug products were indistinguishable by sight, taste, and smell to obviate potential unblinding or patient bias. The patients were trained to empty the sachet and stick-pack into approximately 200 mL of cool (or room temperature) water, stir the powders until dissolved, and drink the contents during the first half of each meal.

Blinding

The randomization code, treatment assignments, and kit allocations were administered by Perceptive eClinical (Nottingham, UK).

Upper Gastrointestinal Endoscopy and Duodenal Biopsy

Upper GI endoscopy and duodenal biopsy were performed by qualified and experienced endoscopists. Standardized procedures were followed for sedation, gastroscopy, and biopsy. The upper GI endoscopy was performed using a video gastroscope at baseline, at week 12 (and at week 24 for those patients participating in study period 3), or early termination. All significant macroscopic abnormalities noted in the esophagus, stomach, duodenal bulb, and descending duodenum were documented. Details of the procedure and processing of the

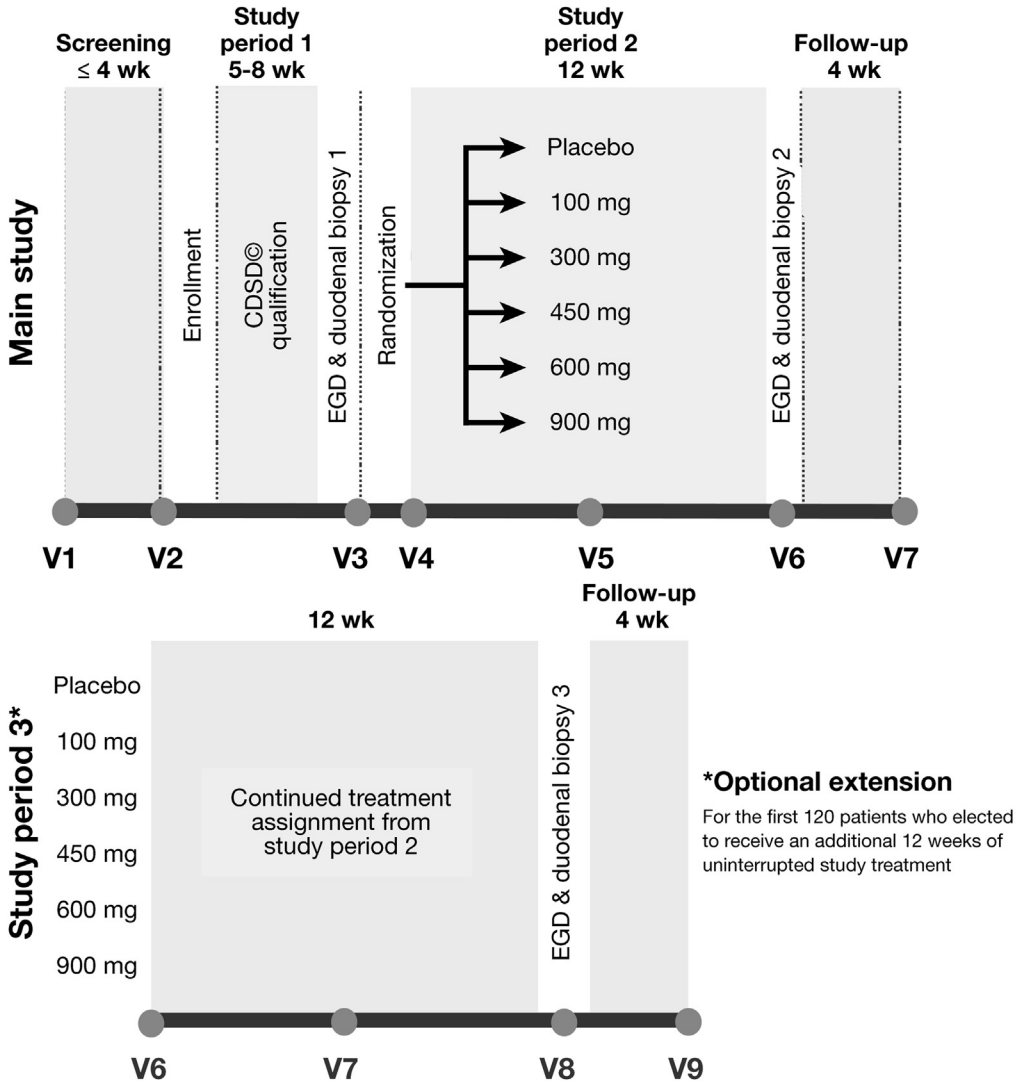

Figure 1. The study design consisted of an initial 4-week screening period. Once patients were recruited, there was an approximate 5-week period during which patients were not administered any medication, but recorded their symptoms. Patients whose symptoms reached the predetermined threshold then underwent endoscopy. Patients who met the threshold of a Vh:Cd ratio of 2 or less then were randomized into the active 12-week study. This was followed by a 4-week placebo run-out period. The first approximately 120 patients were recruited to have a 12-week extension so their participation in active treatment was 24 weeks. EGD, esophagogastroduodenoscopy.

biopsy specimens is described in the Supplementary Materials and Methods section: Core Histology Laboratory Processing and Reading of Duodenal Biopsy Samples.

The primary clinical efficacy assessment was performed using the formalin-fixed H&E-stained specimens. Morphometric analysis of the small-bowel biopsy specimens involved measurement of villous heights and crypt depths, and calculation of their ratios. The Vh and Cd measurements were obtained from well-oriented biopsy samples as previously described.[27–30] These measurements were performed at multiple loci so as not to miss patchy forms of villous atrophy. The average ratio of Vh:Cd was determined from 5 to 12 properly oriented biopsy villous-crypt units. Vh:Cd measurements were performed on baseline biopsy specimens (beginning of study period 1), at the end of the 12-week study treatment (study period 2), and at the end of 24 weeks for those patients participating in study period 3.

Celiac Serology

Whole blood for celiac serology was collected and analyzed at screening, at day 1, and at week 12 (also at week 24 for those patients participating in study period 3). Antibodies (TG2-IgA, DGP-IgA, and DGP-IgG) were measured by an enzyme-linked immunosorbent assay (QUANTA Lite h-tTGIgA, QUANTA Lite Gliadin IgA II, QUANTA Lite Gliadin IgG II, INOVA Diagnostics, Inc, San Diego, CA). DGP-IgG antibodies were measured by enzyme-linked immunosorbent assay to detect antibody titer changes in potential IgA-deficient individuals.

PRO

The PRO instruments used in this study consisted of the CDSD, Impact of Celiac Disease Symptoms Questionnaire (ICDSQ) (ImmunogenX, Asheville, NC), Patient Global Impression-Symptoms (PGI-S), and short form 12 (SF-12) v2

Health Survey. The ICDSQ, PGI-S, and SF-12 v2 Health Survey questionnaires were completed before all other evaluations during a site visit.

CDSD

The CDSD was used to assess symptoms daily. The presence or absence of a symptom in the prior 24-hour period was first established. If the symptom was present (ie, endorsed), follow-up questions were presented to establish severity. Higher scores indicated greater symptom severity.

Symptom qualification required patients who had at least 1 moderate or severe symptom (ie, diarrhea, constipation, abdominal pain, bloating, nausea, or tiredness) contained in the CDSD during days 15–28; patients who experienced tiredness also needed to experience at least 1 GI-related symptom to qualify for study enrollment. The severity qualification of each CDSD symptom was defined as described in Supplementary Table 1.

ICDSQ

The ICDSQ is a quality-of-life instrument designed to assess the impact of patients' celiac symptoms over the previous week at baseline, week 6, and week 12 (and at weeks 18 and 24 for those patients participating in study period 3). The questionnaire comprises 14 items with 4 domains: daily activities (4 items), social activities (3 items), emotional well-being (5 items), and physical functioning (2 items). Each item had 5 response options ranging from "not at all" to "completely." The domains were scored separately and an equally weighted overall impact score also was calculated.

PGI-S

The PGI-S was used to assess change over time in symptom severity and impact of symptoms at baseline, week 6, and week 12 (and at weeks 18 and 24 for those patients participating in study period 3). The first PGI-S item asked patients to rate their symptom severity over the previous 7 days on a 6-point rating scale from "no" to "very severe" symptoms. For those patients reporting the presence of symptoms, the second PGI-S item asked patients to rate the extent to which their celiac symptoms had a negative impact on their daily activities, social activities, emotional well-being, and physical functioning using a 5-point rating scale from "not at all" to "completely."

SF-12 v2 Health Survey

The SF-12 v2 Health Survey asked patients to answer 12 questions that measured physical and mental health at baseline, week 6, and week 12 (and at weeks 18 and 24 for those patients participating in study period 3).

Safety Assessments

Patient safety was monitored by recording non-serious and serious adverse events (AEs). Once patients were randomized, all AEs were reported; classic celiac disease–associated symptoms were assessed by the study physicians as being related either to study treatment or procedure (endoscopy). Additional safety assessments included physical examination, vital signs (blood pressure, heart rate, temperature), electrocardiogram and serum chemistries, hematology, and urinalysis.

Statistical Analysis

The primary efficacy end point was the change from baseline at week 12 in intestinal mucosal morphometry (Vh:Cd). The primary efficacy analysis was performed in the modified intent-to-treat (MITT) population using an analysis of covariance (ANCOVA) model. The MITT population included all randomized patients who were on study treatment for at least 6 weeks, 80% or more compliant with study treatment during the first 12 weeks of study treatment, and had a post-treatment observation of the analysis parameter performed within 14 days of the last study treatment during the first 12 weeks. For the ANCOVA model, the dependent variable was the change from baseline at week 12 in Vh:Cd and the model included effects for treatment group, baseline serology status, and the baseline value of Vh:Cd. If the week 12 value was missing, the patient was not included in any analysis for that parameter. The treatment extension MITT population (ie, patients who received 24 weeks of treatment) was assessed similarly to those receiving 12 weeks of treatment.

The secondary efficacy end points of change from baseline at week 12 in IELs, serology titers, and CDSD were analyzed using similar types of ANCOVA models as described for the primary efficacy end point. All secondary analyses were conducted using 2-sided tests at the $\alpha = .05$ level of significance. Change from baseline within a treatment group was tested using a t test.

These analyses were performed on MITT (for time points: baseline and week 12) and treatment extension MITT populations (for the time points: baseline, week 12, and week 24).

For continuous variables, descriptive statistics included the number of patients reflected in the calculation (n), mean and SD, median, minimum, and maximum. Categoric variables were summarized using frequency counts and percentages. Missing data were not imputed.

Sample Size

Based on the results of prior studies, the SD of the Vh:Cd change from baseline to week 12 was estimated to be in the range from 0.45 to 0.70. For an SD of 0.70, and under the assumption that approximately 90% of randomized patients would be included in the MITT population, the primary comparison between the placebo group and the 600-mg group would have 87% power to detect a treatment difference of 0.3, based on the use of a 2-sided test at the $\alpha = .05$ level of significance. For comparisons between 2 latiglutenase groups combined vs placebo (with a total sample size of 150 patients in the latiglutenase combined group), there would be 93% power to detect a treatment difference of 0.3.

Ethics

The study was conducted in accordance with the International Council on Harmonization guideline E6 Good Clinical Practice, the Declaration of Helsinki, European Union Directives, and with applicable local regulations governing clinical trials. The principal investigators agreed to adhere to the basic principles of Good Clinical Practice, as outlined in 21 Code of Federal Regulations (CFR) 312, Subpart D, Responsibilities of

Sponsors and Investigators, 21 Code of Federal Regulations, Part 50, 1998, and 21 Code of Federal Regulations, Part 56, 1998. All patients provided written informed consent. This is a registered clinical trial (https://clinicaltrials.gov/ct2/show/NCT01917630) and all authors had access to the data, and reviewed and approved the final manuscript.

Results

Study Population

Between August 26, 2013, and December 12, 2014, there were 1919 patients screened for study eligibility. As shown in Supplementary Figure 1, there were 1575 patients entered in study period 1, and 494 patients were randomized and entered the treatment phase of the study (study period 2). The majority of randomization failures (77%) were because the biopsy did not meet the randomization eligibility criteria of Vh:Cd of 2.0 or less. A total of 412 patients (83.4%) completed the study through week 12; 118 eligible patients in the United States enrolled in study period 3, although 2 patients did not receive study medication during the treatment extension, and 108 patients (93.1%) completed the study through week 24. The last patient completed the last study visit (follow-up visit) on May 28, 2015.

The majority of the study population was female (78%). Across treatment groups, there were greater percentages of females in the placebo group (83.1%) and the 900-mg dose group (87.2%) compared with other dose groups (range, 68%–79.2%). Conversely, there were greater percentages of males in the 100-mg dose group (32%) and 600-mg dose group (29.3%) compared with other dose groups (range, 12.8%–22.7%). Compared with the placebo group (mean age, 50.1 y), the 600-mg dose group (mean age, 48.1 y) and the 900-mg dose group (mean age, 47.6 y) were slightly younger, although the age ranges were comparable across all treatment groups. All other demographics and baseline characteristics were comparable across treatment groups, except the mean Vh:Cd at eligibility was slightly higher in the 900-mg dose group (1.51) compared with other dose groups (range, 1.39–1.46).

Demographic and baseline characteristics for the primary efficacy (Vh:Cd) MITT population and the MITT treatment extension populations are provided in Table 1 and Supplementary Table 3, respectively. Overall, the demographic and baseline characteristics of the MITT population and the MITT treatment extension population were similar. A greater percentage was female in both populations across treatment groups. In the MITT treatment extension population, there was a greater portion of males

Table 1. Demographic and Baseline Characteristics: Modified Intent-to-Treat Population

	Treatment group (n = 405)					
	Placebo (n = 125)	100 mg (n = 47)	300 mg (n = 77)	450 mg (n = 39)	600 mg (n = 80)	900 mg (n = 37)
Sex, n (%)						
Male	21 (16.8)	13 (27.7)	22 (28.6)	7 (17.9)	24 (30.0)	2 (5.4)
Female	104 (83.2)	34 (72.3)	55 (71.4)	32 (82.1)	56 (70.0)	35 (94.6)
Age, *y*						
Mean (SD)	50.6 (13.59)	51.5 (13.74)	52.8 (14.34)	51.2 (11.83)	48.5 (12.93)	48.5 (12.90)
Median	51.9	51.9	55.6	51.0	50.4	50.3
Range	23.1–79.6	20.4–75.2	20.6–75.7	28.4–75.5	22.3–75.7	25.1–71.6
Ethnicity, n (%)						
Hispanic	2 (1.6)	3 (6.4)	1 (1.3)	0	2 (2.5)	0
Non-Hispanic	123 (98.4)	44 (93.6)	76 (98.7)	39 (100)	78 (97.5)	37 (100)
Race, n (%)						
White	121 (96.8)	44 (93.6)	77 (100)	38 (97.4)	77 (96.3)	35 (94.6)
Black or African American	1 (0.8)	0	0	0	0	0
Asian	0	1 (2.1)	0	0	0	1 (2.7)
American Indian or Alaskan Native	1 (0.8)	1 (2.1)	0	1 (2.6)	1 (1.3)	1 (2.7)
Other	2 (1.6)	1 (2.1)	0	0	2 (2.5)	0
Height, *cm*						
Mean (SD)	166.9 (8.97)	167.0 (9.15)	167.8 (9.62)	166.0 (7.10)	168.8 (8.85)	162.5 (10.39)
Median	166.4	167.6	167.6	165.1	168.2	162.6
Range	144.8–200.7	149.9–185.4	151.1–188.0	153.7–182.9	153.6–194.5	121.9–185.4
Body mass index, kg/m^2						
Mean (SD)	27.8 (5.39)	27.9 (6.25)	26.6 (5.24)	28.1 (5.74)	27.2 (5.85)	26.8 (4.43)
Median	26.8	26.7	26.5	26.9	26.4	26.5
Range	18.7–44.5	17.0–47.0	17.9–43.8	20.1–47.9	19.0–47.8	19.1–36.5
Vh:Cd eligibility						
Mean (SD)	1.44 (0.498)	1.45 (0.385)	1.36 (0.503)	1.41 (0.487)	1.38 (0.548)	1.48 (0.498)
Median	1.60	1.40	1.50	1.50	1.50	1.70
Range	0.00–2.00	0.40–2.00	0.00–2.00	0.20–2.00	0.00–2.00	0.10–2.00

(40.9%) to females (59.1%) in the 300-mg dose group compared with the portion of males (28.6%) to females (71.4%) in the MITT population. Age ranges were comparable across treatment groups in both populations; however, in the MITT treatment extension population, the 300-mg dose group (mean age, 57.2 y) was older and the 450-mg dose group (mean age, 47.3 y) was younger compared with other treatment groups in both the MITT and MITT treatment extension populations.

The mean Vh:Cd at eligibility (range, 1.16–1.46) was slightly lower across treatment groups in the MITT treatment extension population compared with the mean Vh:Cd at eligibility (range, 1.36–1.48) in the MITT population. Within populations there were no differences across treatment groups.

Overall, the demographic and baseline characteristics of the safety population and the MITT population across treatment groups showed similar patterns, except the 900-mg dose group in the MITT population (94.6%) was predominantly female compared with the safety population (87.2%).

Efficacy End Points

The primary experimental hypothesis for this study was an expected treatment difference in the change in Vh:Cd from baseline to post-treatment; secondary end points included the changes in CD3+ IEL cell densities from duodenal mucosal biopsy specimens, and TG2-IgA, DGP-IgA, and DGP-IgG antibody titers.

In the MITT and treatment extension MITT populations, the primary comparison of change from baseline in Vh:Cd, CD3+ IEL, TG2-IgA, DGP-IgA, DGP-IgG at weeks 12 or 24 did not show superiority of the active treatment groups when compared with placebo (Table 2 and Supplementary Tables 4–21) (week 12 CD3+ IELs and serology analyses as well as all week 24 analyses). No subpopulations of patients were identified who showed superiority of the active treatment arms compared with placebo (Table 3). There was a statistically significant, but clinically trivial, greater effect of placebo on Vh:Cd than on the treatment groups.

Symptoms and Quality of Life

The CDSD and ICDSQ were developed as celiac disease–specific PRO instruments. Details of the development and psychometric performance of these instruments will be published elsewhere. These instruments were used in this clinical study to determine the effect of different dose levels of latiglutenase at 12 and 24 weeks on celiac disease symptom frequency and severity (CDSD) and quality of life (ICDSQ).

A descriptive summary of scores shows that many symptoms occurred less frequently over time for patients receiving one of the dose levels of latiglutenase treatment; however, these results were observed similarly among placebo-treated patients. The results from the ANCOVA models suggest that, for some measures, differences between patients treated with latiglutenase appear to be trending in the direction of having less symptom frequency and severity than observed in the placebo-treated patients (Supplementary Table 22).

To define clinically meaningful responses, an anchor-based minimum important difference was estimated based on the PGI-S improvement category. Although clinically meaningful differences from placebo-treated patients were not observed for any combination of treatment groups for any of the CDSD frequency or severity scores, with the

Table 2. Summary of Villous Height to Crypt Depth Ratio in the (Week 12) Modified Intent-to-Treat Population

	Treatment group (N = 405)					
Vh:Cd	Placebo (N = 125)	100 mg (N = 47)	300 mg (N = 77)	450 mg (N = 39)	600 mg (N = 80)	900 mg (N = 37)
Baseline						
Patients, n	125	47	77	39	80	37
Mean (SD)	1.66 (0.504)	1.67 (0.432)	1.62 (0.541)	1.68 (0.430)	1.61 (0.566)	1.74 (0.478)
Median	1.80	1.72	1.77	1.78	1.64	1.82
Range	0.00–2.49	0.61–2.43	0.00–2.44	0.55–2.38	0.00–2.67	0.25–2.49
Week 12						
Patients, n	125	47	77	39	80	37
Mean (SD)	1.93 (0.592)	1.79 (0.438)	1.77 (0.621)	1.73 (0.609)	1.74 (0.665)	1.86 (0.599)
Median	1.98	1.85	1.82	1.87	1.77	1.85
Range	0.06–3.28	0.51–2.84	0.00–3.12	0.39–2.75	0.06–3.62	0.28–2.81
Change from baseline at week 12						
Patients, n	125	47	77	39	80	37
Mean (SD)	0.27 (0.401)	0.12 (0.463)	0.15 (0.413)	0.05 (0.501)	0.14 (0.493)	0.11 (0.460)
Median	0.30	0.10	0.17	0.15	0.09	0.17
Range	−0.65 to 1.32	−0.88 to 1.85	−0.80 to 1.28	−1.18 to 0.77	−0.77 to 1.72	−0.87 to 1.48
P value	<.0001	.0805	.0018	.5560	.0157	.1449

NOTE. These *P* values reflect the intragroup change between baseline and follow-up evaluation, not a comparison between treatment groups.

Table 3. Analysis of Covariance of the Change From Baseline of Villous Height to Crypt Depth Ratio in the (Week 12) Modified Intent-to-Treat Population

	Baseline		Week 12 change from baseline			Week 12 treatment difference[a]		
Vh:Cd	N	Mean	N	Mean	95% CI	LS mean	95% CI	*P* value
Placebo	125	1.66	125	0.27	0.20–0.35			
100 mg	47	1.67	47	0.12	−0.02 to 0.26			
300 mg	77	1.62	77	0.15	0.06–0.25			
450 mg	39	1.68	39	0.05	−0.11 to 0.21			
600 mg	80	1.61	80	0.14	0.03–0.25			
900 mg	37	1.74	37	0.11	−0.04 to 0.27			
Treatment effect[b]								
600 mg + 900 mg vs placebo						−0.15	−0.264 to −0.039	.0084
600 mg vs placebo						−0.15	−0.273 to −0.031	.0136
600 mg + 450 mg vs placebo						−0.19	−0.301 to −0.078	.0009
300 mg + 450 mg vs placebo						−0.18	−0.289 to −0.065	.0020
300 mg vs placebo						−0.13	−0.248 to −0.004	.0425
300 mg + 100 mg vs placebo						−0.14	−0.249 to −0.032	.0113

CI, confidence interval; LS, least square.
[a]Difference between treatments for the least square mean change from baseline, obtained from the model.
[b]Treatment effect *P* value was based on an ANCOVA model: change = treatment + baseline + baseline serology status.

exception of constipation, the CDSD showed a reduction in symptom frequency and severity for improvement and an increase for deterioration in PGI-S category (Supplementary Table 23). The CDSD appeared to be sensitive to these categoric gradations of improvement and decline. Future additional analyses could be performed evaluating clinically meaningful differences based on serologic status; these analyses were not performed because of the overall lack of a clear therapeutic effect of the active treatment on more objective measures of histologic or serologic responses to active treatment.

Another objective of this study was to evaluate the effect of different dose levels of latiglutenase on quality of life at 12 and 24 weeks as measured by the ICDSQ, the SF-12v2, and the SF-6D. No clinically meaningful changes from baseline over placebo were observed among any treatment group combinations for any of the instruments at weeks 12 or 24 (Supplementary Tables 24–31). An assessment of potential correlations between clinical end points (ie, histologic or serologic measures) was performed; correlations between CDSD scores and clinical outcomes were weak.

Although the trial was not designed to evaluate specific performance of the quality-of-life instruments, the ICDSQ performed well based on the available data. Consistent with the reported CDSD symptom frequency and severity results, the impacts for each domain (daily activities, social activities, emotional well-being, and physical function) and the overall score showed that the full range of scores was reported. For example, significant improvement in symptoms over time was reflected appropriately in all ICDSQ domains as well as in the overall score.

Study Treatment Compliance

Overall, compliance rates were quite high, with rates of 94.4% or greater across treatment groups. Patients across all treatment groups who participated in the treatment extension were 100% compliant during the first 12 weeks of the study (study period 2); compliance rates remained high during the treatment extension (study period 3), with 100% compliance rates in all treatment groups except the 300-mg dose group, in which the compliance rate was 96%.

Adverse Events

GI disorders and infections by system organ class were the most frequently reported adverse events, although no single GI or infection event was reported with any significant frequency. Few of these events were attributed to study medication and most events were mild to moderate in severity. An overall summary of AEs by treatment group at weeks 12 and 24 are provided in Tables 4 and 5, and the most frequently reported adverse effects in the safety and treatment extension populations are shown in Supplementary Table 32. In the safety population, GI disorders were reported in 24%–31.9% of patients across treatment groups with no difference from placebo (31.1%); no dose effect was observed in the frequency of these events. No single GI event was reported with any significant frequency. Few of these events were attributed to study medication and most events were mild to moderate in severity. Infections were reported in 14.0%–27.3% of patients across treatment groups with no difference from placebo (24.3%); no dose effect was observed in the frequency of these events. No single infection event was reported with any significant frequency. Only 1 event (fungal infection in the 600-mg dose group) was attributed to study medication and was moderate in severity.

A similar pattern of AEs was observed in the treatment extension population, in which GI disorders and infections were the most frequently reported adverse effects. GI disorders were reported in 8.3%–36.8% of patients across

Table 4. Summary of Adverse Events in the (Week 12) Safety Population

	Treatment group (n = 489)					
	Placebo (n = 148)	100 mg (n = 50)	300 mg (n = 97)	450 mg (n = 48)	600 mg (n = 99)	900 mg (n = 47)
Patients with any AEs	83 (56.1%)	24 (48.0%)	55 (56.7%)	22 (45.8%)	54 (54.5%)	21 (44.7%)
Patients with AEs related to study medication						
Related	26 (17.6%)	6 (12.0%)	17 (17.5%)	13 (27.1%)	15 (15.2%)	11 (23.4%)
Not related	56 (37.8%)	18 (36.0%)	38 (39.2%)	9 (18.8%)	39 (39.4%)	10 (21.3%)
Not applicable	1 (0.7%)	0	0	0	0	0
Patients with AEs related to study procedure						
Related	2 (1.4%)	2 (4.0%)	1 (1.0%)	0	1 (1.0%)	0
Not Related	81 (54.7%)	22 (44.0%)	54 (55.7%)	22 (45.8%)	53 (53.5%)	21 (44.7%)
Patients with AEs by maximum severity						
Mild	21 (14.2%)	8 (16.0%)	18 (18.6%)	11 (22.9%)	17 (17.2%)	5 (10.6%)
Moderate	43 (29.1%)	13 (26.0%)	26 (26.8%)	7 (14.6%)	24 (24.2%)	9 (19.1%)
Severe	19 (12.8%)	3 (6.0%)	11 (11.3%)	4 (8.3%)	13 (13.1%)	7 (14.9%)
Patients with study medication–related AEs by maximum severity						
Mild	10 (6.8%)	3 (6.0%)	5 (5.2%)	7 (14.6%)	6 (6.1%)	3 (6.4%)
Moderate	12 (8.1%)	3 (6.0%)	8 (8.2%)	3 (6.3%)	6 (6.1%)	4 (8.5%)
Severe	4 (2.7%)	0	4 (4.1%)	3 (6.3%)	3 (3.0%)	4 (8.5%)
Patients with study procedure–related AEs by maximum severity						
Mild	0	2 (4.0%)	0	0	0	0
Moderate	2 (1.4%)	0	1 (1.0%)	0	0	0
Severe	0	0	0	0	1 (1.0%)	0
Patients with serious AEs	3 (2.0%)	0	1 (1.0%)	0	0	1 (2.1%)
Patients with serious AEs related to study medication	0	0	0	0	0	0
Patients with serious AEs related to study procedure	0	0	0	0	0	0

Table 5. Summary of Adverse Events in the Treatment Extension (Week 24) Safety Population

	Treatment group (n = 116)					
	Placebo (n = 33)	100 mg (n = 14)	300 mg (n = 25)	450 mg (n = 12)	600 mg (n = 19)	900 mg (n = 13)
Patients with any AEs	22 (66.7%)	7 (50.0%)	16 (64.0%)	3 (25.0%)	16 (84.2%)	6 (46.2%)
Patients with AEs related to study medication						
Related	4 (12.1%)	2 (14.3%)	1 (4.0%)	1 (8.3%)	4 (21.1%)	0
Not related	18 (54.5%)	5 (35.7%)	15 (60.0%)	2 (16.7%)	12 (63.2%)	6 (46.2%)
Patients with AEs related to study procedure						
Related	1 (3.0%)	0	0	0	0	0
Not related	21 (63.6%)	7 (50.0%)	16 (64.0%)	3 (25.0%)	16 (84.2%)	6 (46.2%)
Patients with AEs by maximum severity						
Mild	6 (18.2%)	3 (21.4%)	5 (20.0%)	2 (16.7%)	5 (26.3%)	0
Moderate	13 (39.4%)	4 (28.6%)	10 (40.0%)	0	9 (47.4%)	3 (23.1%)
Severe	3 (9.1%)	0	1 (4.0%)	1 (8.3%)	2 (10.5%)	3 (23.1%)
Patients with study medication–related AEs by maximum severity						
Mild	1 (3.0%)	2 (14.3%)	1 (4.0%)	1 (8.3%)	2 (10.5%)	0
Moderate	3 (9.1%)	0	0	0	2 (10.5%)	0
Severe	0	0	0	0	0	0
Patients with study procedure–related AEs by maximum severity						
Mild	0	0	0	0	0	0
Moderate	1 (3.0%)	0	0	0	0	0
Severe	0	0	0	0	0	0
Patients with serious AEs	0	0	1 (4.0%)	0	0	1 (7.7%)
Patients with serious AEs related to study medication	0	0	0	0	0	0
Patients with serious AEs related to study procedure	0	0	0	0	0	0

treatment groups with no difference from placebo (36.4%); no dose effect was observed in the frequency of these events. No single GI event was reported with any significant frequency. Few of these events were attributed to study medication and most events were mild to moderate in severity. Infections were reported in 15.4%–47.4% of patients across treatment groups with no difference from placebo (33.3%); no dose effect was observed in the frequency of these events. No single infection event was reported with any significant frequency.

Early Withdrawals

Thirty-six patients withdrew from the study because of AEs; 5 of the 36 patients withdrew from the study during the treatment extension. GI disorders were the most frequent AEs leading to study discontinuation across treatment groups (placebo, 6 of 9 patients; 100 mg, 0 of 2 patients; 300 mg, 7 of 9 patients; 450 mg, 4 of 4 patients; 600 mg, 7 of 7 patients; and 900 mg, 4 of 5 patients). Most of these GI events were moderate to severe in severity and were considered to be possibly related to study treatment.

Serious Adverse Events

Five serious AEs were reported after randomization in the study: hypoxic respiratory failure (300-mg dose group), optic neuritis (placebo dose group), acute pancreatitis (900-mg dose group), cholelithiasis (placebo dose group), and worsening of paroxysmal atrial fibrillation (placebo dose group); none was considered by the investigators to be related to study treatment.

Discussion

This study of a potential therapeutic intervention in patients with symptomatic celiac disease and evidence of mucosal injury is remarkable in its design, rigor, size, and outcome. The study was designed to test the hypothesis that the experimental drug latiglutenase would show greater improvement in histologic and symptomatic measures of disease activity than placebo-treated patients in a celiac disease population experiencing ongoing moderate or severe symptoms and showed histologic evidence of significant duodenal mucosal injury despite following a GFD. The results of the study did not support the original hypothesis. All study groups including placebo had a substantial significant improvement in both histologic scores and symptoms. Unique to this study are the insights, afforded by the rigorous collection of data, into the spectrum of symptoms and adverse events experienced over time by patients with celiac disease following a GFD.

It is important to try to understand the potential cause(s) of the study results to inform any future decisions regarding development of other potential therapeutics. Major considerations include the following: variability in the primary end point, a strong trial effect, insufficient duration of study treatment to show positive remodeling of the duodenal mucosa, core histology laboratory variability, noncompliance with study medication, study medication instability, symptomatic relief leading to patients liberalizing gluten intake, and other factors that could not be controlled. The potential causes that may have affected the study results are discussed later.

Data Reliability

Every effort was made to minimize patient-to-patient variability and patients were instructed repeatedly to maintain their usual GFD throughout the entire study. The prolonged run-in period (28 days) when patients reported symptoms daily while receiving neither placebo nor active study drug established a reliable assessment of baseline symptoms because only those experiencing moderate or severe symptoms during days 15–28 proceeded to duodenal biopsy and quantification of the severity of mucosal injury. A single core histology laboratory facilitated uniform processing and interpretation. Interpreter bias was avoided further by pairing pretreatment and post-treatment (week 12) biopsy specimens in random sequence and in groups of 10 biopsy specimens with reading of Vh:Cd and CD3+ IEL by a single pathologist. Week 24 biopsy specimens were read at the time they were available; however, the pathologist was blinded to treatment group or timing of sample collection. All clinical sites were trained on all study procedures and all endoscopists were instructed on the study-specific biopsy sample collection standards and procedures. All of these efforts resulted in the generation of a reliable data set with all controllable sources of variability minimized.

Potential Trial Effect Leading to Greater Adherence to the GFD

All patients were reminded to maintain their current gluten-free habits; however, the patient training and the medication preparation required the study medication to be taken 3 times daily with each major gluten-free meal, which may have been a continual reminder of the patient's need to adhere to a gluten-free diet. Although the presentation of the study treatment avoided the chances of inadvertent unblinding by the patients, it may have drawn attention to disease status, which could have resulted in improved gluten-avoidance behavior. Patients also were instructed to complete the CDSD each evening (using a telephone touchpad to record their answers), after their last meal of the day and before going to bed. Patients who had not completed their diary by 8 PM local time received an automated telephone call or text reminder to fill out the diary. Each of these instructions may have unintentionally affected patient behavior and improved the level of adherence to the GFD.

The patients randomized into this study had eligibility Vh:Cd ratios that ranged from 0.0 to 2.0; such values are consistent with those seen in patients with celiac disease who do not adhere to a GFD. Estimates of the amount of daily gluten these patients were consuming can be made by reviewing data from 2 previous studies.[27] In those studies, patients with celiac disease were administered 1.5, 2.0, 3.0, and 6.0 g of gluten daily for 6 weeks and quantitative mucosal morphometric responses were measured. The

gluten dose-response curves suggest that patients with Vh:Cd values of 2.0 or less may have been consuming considerable amounts of gluten (2–6 g/day or more) in their diets despite their stated efforts to follow a GFD. An alternative explanation could be that the patients randomized into this study were sensitive to even minute quantities of gluten. This is unlikely because the mucosal responses of patients observed in this study resembled the magnitude of mucosal morphometric improvement of published responses of previously untreated, newly diagnosed patients starting a GFD.[31]

There is also the somewhat perverse possibility that a positive treatment effect could be counteracted by a reciprocal increase in gluten intake. In the higher-dose groups (eg, 600 and 900 mg compared with placebo), there appeared to be a greater effect on the frequency and severity of abdominal pain, bloating, and tiredness (see discussion later). This improvement potentially could have resulted in patients in those dose groups being able to tolerate increased dietary gluten intake without harm and therefore become less rigorous in their adherence to the GFD.

The putative mechanism of action of latiglutenase is the degradation of the proline and glutamine-rich immunogenic peptides found in gluten. The substrate of latiglutenase is gluten, and therefore its therapeutic activity depends on the presence of substrate in stomach at the time of administration. If the amount of gluten in the stomach at the time of study treatment administration was very small to absent, then it would not be possible for the study treatment to show an effect. In support of this hypothesis are the results of a gluten-challenge study in patients with well-controlled celiac disease (ie, seronegative with minimal to no symptoms) who were administered a daily gluten challenge for 6 weeks.[27] In that study, latiglutenase was shown to attenuate gluten-induced mucosal injury in patients. A major difference between that study and the current one is that in the gluten-challenge study, there was a defined amount of substrate (gluten) in the stomach when latiglutenase was administered.

When designing the current study, we only included patients with screening baseline Vh:Cd of 2.0 or less, with the expectation that the presence of an injured mucosa was the result of the consumption of gluten, which could serve as substrate for the study treatment. However, if a trial effect resulted in greater adherence to a GFD, then the potential beneficial effects of latiglutenase would be diminished or possibly eliminated by the reduction or removal of the substrate from the diet. Finally, because the biologic target of latiglutenase is not a host cellular target, the drug could not be expected to show a disease-modifying effect in the absence of ongoing consumption of gluten.

It is unlikely that the study treatment duration (12 weeks for all patients, 24 weeks for 108 patients) was insufficient to show histologic improvement because 83 patients showed an improvement in Vh:Cd of 0.5 or greater by week 12, further supporting the hypothesis that the mucosal responses in many of the patients studied were responding to the withdrawal of most of the gluten in their diets.

Another potential source of variability that could affect the outcome of the study was the histologic measurements performed by the Core Histology Laboratory; however, it is not likely that this was the case. Extensive measures were taken to minimize the potential for variability introduced by reading the histologic specimens. All biopsy specimens were delivered overnight in buffered formalin to the Central Core Histology Laboratory for processing, embedding, cutting, staining, and reading. For the primary end point, all baseline samples from randomized patients were archived and read randomly at the time when a paired week-12 post-treatment sample was available; all paired samples were read by only 1 pathologist to eliminate interobserver variability, which was determined to be 0.22 (data not shown), which was lower than commonly reported ($\geq$0.3).[27] Thus, it is unlikely that the variability in the readout of the Vh:Cd or CD3+ IELs was a significant factor affecting the outcome of the study.

Patient compliance with study treatment was excellent; compliance rates averaged 94.4% across treatment groups, and thus is an unlikely explanation for the study outcome. This shows that patients with celiac disease are highly compliant and readily can take a meal-timed medicine, at least in the study context, with a rate that did not decrease with extension.

The study treatment formulations were tested using in vitro methods at defined intervals throughout the study and shown to be stable for the duration of the study. Thus, drug product instability is unlikely to explain the study outcome.

Several patient-specific factors include the possible consumption of gluten between the major meals or cheating by patients who may have experienced symptomatic improvement while on study, inadequate mixing of study treatment with gluten in the stomach, or preferential gastric emptying of liquids. None of these real-world factors could be controlled for by design of the current study. Whether these factors affected the study outcome is not known. Future studies may benefit from tests of gluten-free diet adherence such as stool urine or blood measures of gluten ingestion.[32]

Although the results did not show clinically or statistically significant differences between the treatment groups and placebo in effect on Vh:Cd, CD3+ IEL, or serologies, latiglutenase appeared to have an effect on some of the symptom domains captured by the CDSD. ANCOVA analysis of the CDSD data showed that the higher doses (600 and 900 mg) of latiglutenase were associated with clinically and statistically significant improvement in the frequency and severity of abdominal pain, bloating, and tiredness, especially in patients who, at baseline, were seropositive (to be published separately). This observation suggests that treatment with latiglutenase may affect symptoms before showing clinically meaningful effects on serologic and histologic end points. Newly diagnosed patients with celiac disease initiated on a GFD first experience an improvement in symptoms (days to weeks), followed by serologic response (months), and lastly by histology (months to years).[9,33,34] These clinical observations are consistent with

the concept that symptoms are the most sensitive indicators of response to gluten withdrawal from the diet.

This study clearly shows that a key variable that will need to be addressed in clinical trials in celiac disease, in which healing of injured mucosa is the overall objective, is the need to design a study that overcomes or at least minimizes the trial effect.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2016.11.004.

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Received April 14, 2016. Accepted November 9, 2016.

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Acknowledgments
The authors wish to acknowledge Tricia L. Brantner, who provided expert coordination of the central pathology laboratory, and Deborah I. Frank, who provided assistance in the preparation of the manuscript.

Members of the CeliAction Study Group of Investigators are as follows: S. Ansari (Longwood Research, Huntsville, AL); K. Ayub (Southwest Gastroenterology, Oak Lawn, IL); A. Basile (Shaw Research Specialists, Miami, FL); C. Behrend (Grand Teton Research Group, Idaho Falls, ID); P. Bercik (McMaster University, Hamilton, Ontario, Canada); B. Bressler (GI Research Institute, Vancouver, British Columbia, Canada); V. Byrnes (University College Hospital, Galway, Ireland); V. S. Chandan (Mayo Clinic, Rochester, MN); V. Cheekati (Agile Clinical Research Trials, Atlanta, GA); B. Chipps (Capital Allergy and Respiratory Disease Center, Roseville, CA); A. Coates (Gastroenterology Associates of Western Michigan, Wyoming, MI); A. Collatrella (Research Protocol Management Specialists, Pittsburgh, PA); J. Condemi (AAIR Research Center, Rochester, NY); C. Corder (COR Clinical Research, Oklahoma City, OK); J. Corren (Allergy Medical Clinic, Los Angeles, CA); C. Curtis (Compass Research, Orlando, FL); M. 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Conflicts of interest
These authors disclose the following: Joseph A. Murray has received grant support from the National Institutes of Health, Alba Therapeutics, the Oberkotter Foundation (Oberkotter #1), and the Broad Medical Research Program at Crohn's & Colitis Foundation of America (342367), has served on the advisory boards of Celimmune, LLC, AMAG Pharmaceuticals, Entera Health, Inc, Sonomaceuticals, LLC, BioLineRx, GlaxoSmithKline, Genentech, and Glenmark Pharmaceuticals Ltd, has served as a consultant to Boehringer Ingelheim, is a patent holder with Miomics, and holds equity options in Torax; Ciarán P. Kelly has received grant support from the National Institutes of Health, has served on the advisory boards of Alvine Pharmaceuticals and Alba Therapeutics, and has served as a consultant for Cour Pharmaceuticals; Peter H. R. Green has provided scientific advice to Alvine Pharmaceuticals, Inc, and has served on the advisory board of ImmusanT; Annette Marcantonio and Daniel C. Adelman are former employees of Alvine Pharmaceuticals, Inc; and Markku Maki has received grant support from Competitive State Research Financing of the Expert Responsibility Area of the Tampere University Hospital (9T040) and Tekes (The Finnish Funding Agency for Innovation 658/31/2015), has served on the advisory boards of the Finnish Celiac Society, Alvine Pharmaceuticals, Inc, ImmusanT, Inc, BioLineRx, Ltd, Celimmune, LLC, and Trivitron Ltd, has served as a consultant to FinnMedi Oy Ltd, GSK Vaccines, Inc, and Glenmark Pharmaceuticals Ltd, is an inventor of the patent "Methods and Means for Detecting Gluten-Induced Diseases," USA patent number 7,361,480-USA, European patent number 1390753, which has been commercialized by FinnMedi Oy Ltd and licensed to Labsystems Diagnostics Ltd. The remaining author discloses no conflicts.

Funding
This study was sponsored by Alvine Pharmaceuticals, Inc, San Carlos, CA. The study sponsor was involved in the design, initiation, supervision, and interpretation of results.

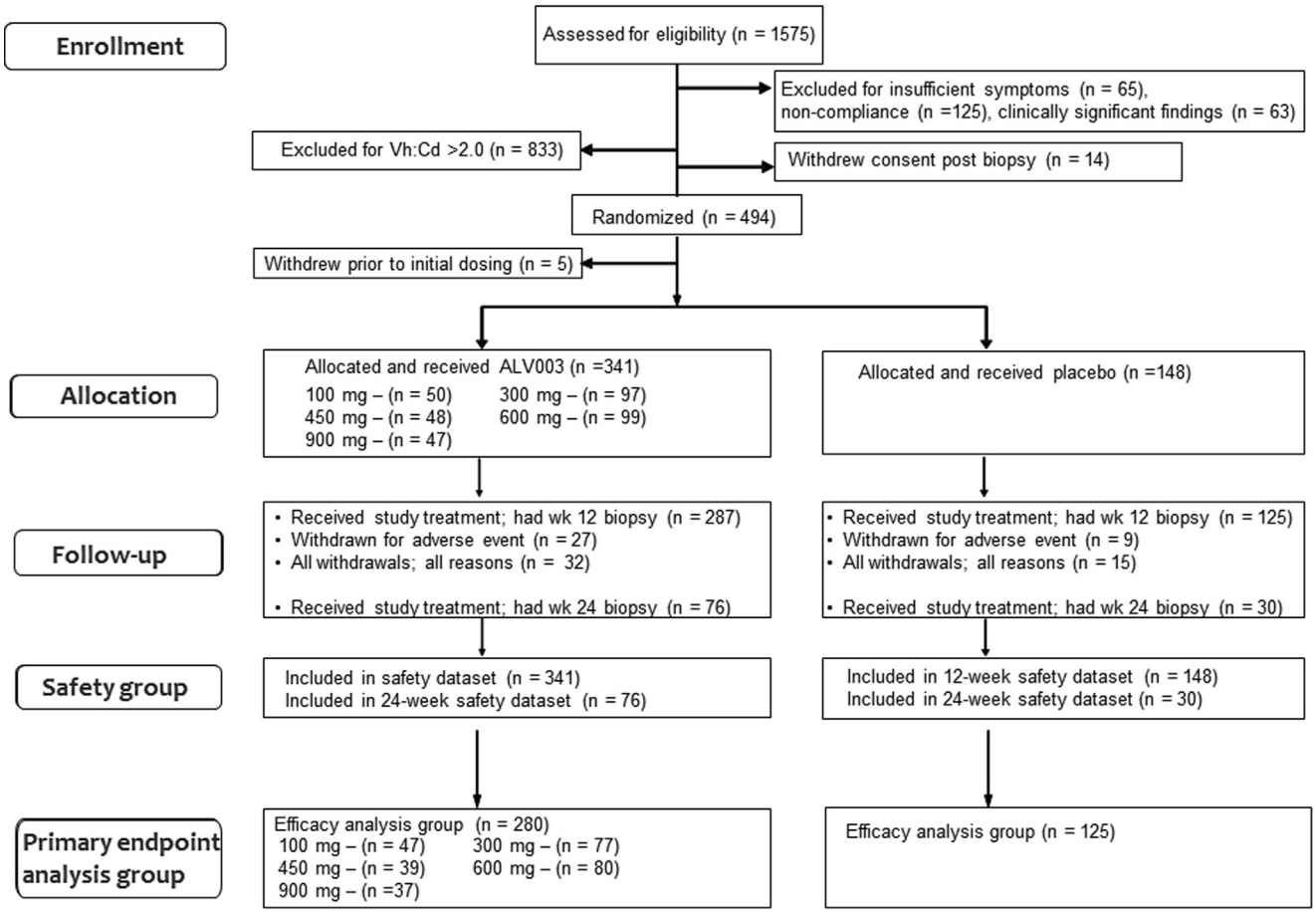
Supplementary Materials and Methods

Core Histology Laboratory Processing and Reading of Duodenal Biopsy Samples

Four small-bowel biopsy specimens were taken from the distal part of the duodenum, placed in vials containing 10% buffered formalin, and sent to the central histopathology core laboratory at Mayo Clinic Pathology (Rochester, MN), where they were embedded in paraffin, oriented, cut, and stained with H&E, and studied under light microscopy. Upon receipt of the week 12 postrandomization biopsy specimen, a single pathologist reader at the Mayo Clinic Core Histology Laboratory performed all paired readings. For patients who had week 24 biopsy specimens, processed and stained specimens were added to a pool of paired samples (ie, baseline and week 12) and were blinded by patient and time of collection. In patients who were eligible for randomization based on eligible Vh:Cd readings of 2.0 or less, duplicate slides were stained for CD3+ IEL quantification and compared with the paired post-treatment specimen. The same pathologist read all baseline and week 12 and 24 specimens.

Verification of Compliance

Patients were instructed to retain all used and unused study medication kits and return them to the clinic at each visit. Patients documented compliance by completing study medication dosing diaries throughout the study treatment phase. Study medication compliance was checked at weeks 2, 6, and 12, and at weeks 18 and 24 for patients who continued into study period 3. At the post-treatment follow-up visit, patients returned all of the used and unused study medication kits and dosing diaries for compliance checks; returned drug was recorded in the drug accountability logs. Site staff recorded any noncompliance and re-trained patients as necessary.



Supplementary Figure 1. Flow diagram for the CeliAction Study.