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Glutenase ALV003 Attenuates Gluten-Induced Mucosal Injury in Patients With Celiac Disease

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BACKGROUND & AIMS: Gluten ingestion leads to small intestinal mucosal injury in patients with celiac disease, necessitating strict life-long exclusion of dietary gluten. Despite adherence to a gluten-free diet, many patients remain symptomatic and still have small intestinal inflammation. In this case, nondietary therapies are needed. We investigated the ability of ALV003, a mixture of 2 recombinant gluten-specific proteases given orally, to protect patients with celiac disease from gluten-induced mucosal injury in a phase 2 trial. **METHODS:** We established the optimal daily dose of gluten to be used in a 6-week challenge study. Then, in the intervention study, adults with biopsy-proven celiac disease were randomly assigned to groups given ALV003 (n = 20) or placebo (n = 21) together with the daily gluten challenge. Duodenal biopsies were collected at baseline and after gluten challenge. The ratio of villus height to crypt depth and densities of intraepithelial lymphocytes were the primary end points. **RESULTS:** A daily dose of 2 g gluten was selected for the intervention study. Sixteen patients given ALV003 and 18 given placebo were eligible for efficacy evaluation. Biopsies from subjects in the placebo group showed evidence of mucosal injury after gluten challenge (mean villus height to crypt depth ratio changed from 2.8 before challenge to 2.0 afterward; $P = .0007$; density of $CD3^+$ intraepithelial lymphocytes changed from 61 to 91 cells/mm after challenge; $P = .0003$). However, no significant mucosal deterioration was observed in biopsies from the ALV003 group. Between groups, morphologic changes and $CD3^+$ intraepithelial lymphocyte counts differed significantly from baseline to week 6 ($P = .0133$ and $P = .0123$, respectively). There were no statistically significant differences in symptoms between groups. **CONCLUSIONS:** Based on a phase 2 trial, the glutenase ALV003 appears to attenuate gluten-induced small intestinal mucosal injury in patients with celiac disease in the context of an everyday gluten-free diet containing daily up to 2 g gluten. Clinicaltrial.gov, Numbers: NCT00959114 and NCT01255696.

Keywords: Duodenal Biopsy; Drug Treatment; Morphometry; Clinical Trial.

Celiac disease is caused by gluten ingestion in a subset of genetically predisposed individuals. Currently, strict, life-long dietary exclusion of gluten is the only option for celiac disease.[1] Prolamins are the main storage proteins found in wheat (gliadin), barley (hordein), and rye (secalin), collectively referred to as dietary gluten. Gluten is responsible for the unique viscoelastic properties of wheat, and accounts for its wide use in different food products.[2] Gluten is added to several noncereal foods, increasing the estimated total amount of gluten in a typical Western diet to 15−20 g daily.[3,4] The prolamins are rich in glutamine and proline residues resulting in incomplete degradation by human gastrointestinal and brush border enzymes[5−7]; these proteins can initiate mucosal inflammation in individuals carrying the HLA-DQ2 or -DQ8.

As a result of incomplete proteolysis, immunogenic peptide fragments of gluten are formed. These peptides are transported through the epithelium and deamidated by tranglutaminase 2 (TG2). The deamidated gliadin peptides are processed by antigen-presenting cells, which bind them to HLA-DQ2 or DQ8 molecules; HLA-bound peptides are presented to antigen-restricted T cells, activating both innate and adaptive immune responses, which in turn culminate in inflammatory injury of the small intestinal mucosa leading to subsequent clinical sequelae.[8]

In most celiac disease patients, initiation of a gluten-free diet (GFD) results in at least partial healing of the duodenal mucosa, improvement in most gluten-associated symptoms, and a decrease in celiac disease−specific antibody titers. However, in many patients, even with long-term strict adherence to a GFD, symptoms, and inflammatory and architectural changes in the small bowel mucosa and positive antibody levels can persist.[9−12] Several factors contribute to incomplete responses to the GFD. Gluten is widely used in the food industry; cross contamination

Abbreviations used in this paper: **AE, adverse event; CDQ, Celiac Disease Questionnaire; DGP, deamidated gliadin peptide; ELISA, enzyme-linked immunosorbent assay; EMA, endomysial antibody; GSRS, gastrointestinal symptom rating scale; IEL, intraepithelial lymphocyte; OCT, optimal cutting temperature; TG2, transglutaminase 2; UGE, upper gastrointestinal endoscopy; VAS, visual analogue scale; VH:CrD, villus height to crypt depth ratio.**

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during food processing is difficult to avoid; the labeling of food products can be inaccurate, misleading, or incorrect; and the GFD is socially troublesome, expensive, and compliance is problematic.[13] Total avoidance of gluten is, at best, challenging. Despite attempted adherence to a GFD, severe villus atrophy in long-term–treated celiac disease patients is frequently observed (reviewed by Ilus et al)[12] It is possible that many patients are consuming hundreds of milligrams (or more) of gluten daily. Taken together, there is a need for the development of a nondietary (pharmacologic) therapy that is either adjunctive to, or a replacement for the GFD.

Possible targets for pharmaceutical intervention are based on understanding the pathogenesis of celiac disease.[14,15] One potential approach to treatment is to degrade the gluten protein into small, nonimmunogenic peptide fragments before they can transit across the small intestinal mucosa. Such gluten-specific proteases, called glutenases, are found in bacteria, fungi, and cereals. Specific enzymes that could function in the gastric environment have been identified, cloned,[16] and pharmacologically characterized.[17,18]

The primary objective of this study was to determine whether ALV003, a novel glutenase could attenuate gluten-induced small intestinal mucosal damage. To do this, we first needed to establish the optimal daily gluten dose that would induce detectable and clinically significant mucosal deterioration over time in celiac disease patients on a GFD. Once the daily gluten dose was identified, we performed a randomized, placebo-controlled, double-blind clinical trial, testing the hypothesis that ALV003, administered daily at the time of gluten ingestion, would protect celiac patients from gluten-induced duodenal mucosal injury, while assessing the tolerability and safety of the active drug.

Patients and Methods

Gluten-Dose Optimization

To determine the optimal dose of gluten to be used in the interventional study, gluten challenges with 1.5 g, 3.0 g, or 6.0 g gluten (using breadcrumbs) were administered daily to adult celiac disease patients for 6 weeks (see Supplementary Material for gluten content assessment). Eligibility criteria included celiac disease diagnosis established by duodenal mucosal biopsy, attempted adherence to a GFD for 1 year or more, and being in clinical remission (ie, TG2-IgA–negative and reporting minimal to no gluten-associated symptoms). Patients were instructed to follow a strict GFD, except for the gluten challenge administered as baked food-grade gluten in the middle of each meal, 3 times each day. Upper gastrointestinal endoscopies with duodenal mucosal biopsies obtained from the descending duodenum were performed at the beginning of the study and at the end of the 6-week gluten challenge to assess histologic and inflammatory marker changes.

Therapeutic Intervention Study

Adult patients (18–75 years old) were enrolled into this randomized, placebo-controlled, parallel-group study. Patients were required to have biopsy-established celiac disease, adherence to a GFD for 1 year or more with minimal or no symptoms, and be TG2-IgA–negative, initially screened by the rapid Biocard Celiac test (Anibiotech, Vantaa, Finland) and confirmed by a serum enzyme-linked immunosorbent assay (ELISA) test (Quanta Lite htTG IgA, Inova Diagnostics, Inc., San Diego, CA). The complete entry criteria are listed in Supplementary Table 1.

After obtaining informed consent, patients were screened by medical history, physical examination, clinical laboratory tests, and electrocardiography. Eligible patients underwent upper gastrointestinal endoscopy (UGE) with duodenal mucosal biopsies; patients with a macroscopically normal UGE at baseline were considered eligible and randomized to receive ALV003 or placebo treatment. The overall schema of the study is shown in Figure 1. At randomization serum for celiac serology tests was obtained (for paired analysis with post-treatment samples); patients were instructed on how to use the study products and complete the symptom-based questionnaires (Gastrointestinal Symptom Rating Scale [GSRS] and Bristol Stool Chart), the quality of life questionnaires (Celiac Disease Questionnaire [CDQ], visual analog scale [VAS], and Short Form-36 version 2), and the meal and drug-dosing diaries. Patients were followed throughout the study for safety by physical examination, clinical laboratory tests, adverse events (AE), and compliance with study treatment administration and

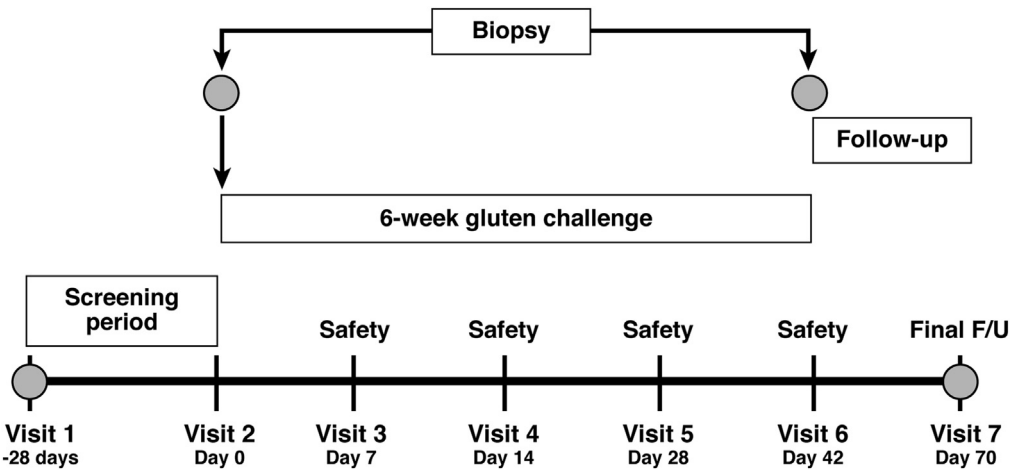

Figure 1. Therapeutic intervention study schema. Patients were randomized to receive ALV003 or a placebo drug. All patients ingested 2 g gluten daily for 6 weeks. Upper gastrointestinal endoscopy with duodenal mucosal biopsies was performed at day 0 (*baseline*) and at post treatment. F/U, follow up.

symptoms. At the end of the gluten-challenge period, patients underwent follow-up UGE with duodenal mucosal biopsies and were instructed to continue their strict GFD. If a patient's participation in the study was discontinued early, the follow-up UGE with duodenal biopsies was performed at the time of discontinuation for patients who had received the gluten challenge for at least 7 days.

Study Treatment

ALV003 consisted of 2 co-administrated gluten-specific proteases, ALV001 and ALV002. ALV001, a modified recombinant version of the proenzyme form of cysteine endoprotease, EP-B2, derived from barley. In vitro studies have shown that ALV001 proteolyzes gluten adjacent to glutamine residues, and ALV002, a modified recombinant version of prolyl endopeptidase from the bacterium *Sphingomonas capsulate* (SC-PEP), proteolyzes the peptide products of ALV001 digestion by cleaving adjacent to proline residues. Together these enzymes degrade gluten more rapidly and thoroughly than either enzyme alone.[19] ALV003 proteolyzes various forms of gluten such as purified gliadin (as well as secalins and hordeins), uncooked gluten flour and whole-wheat bread gluten, eliminating >90% of the immunoreactive epitopes in vitro;[16,19] in vitro doses of ALV003 ≥300 mg degrade >90%−95% of 1.5 g gluten for 30 minutes (data on file). ALV003 is stable at a pH range of 3.5−5 reflecting the post-prandial pH of the stomach, without significant gastrointestinal absorption. The placebo used in the study consisted of the same excipients as those in the ALV003 formulation.

Randomization

Patients were sequentially randomized 1:1 to receive oral treatment with either ALV003 or placebo drug in block sizes of 4 (Prisym Clintrials, version 1.1.1, Prisym ID Limited, Berkshire, UK). All study participants, care providers, data managers, and study personnel remained blinded to study treatment assignment until the analyses were completed. ALV003 900 mg or placebo was administered once daily at one major meal each day; at the same meal, breadcrumbs were consumed orally by all patients. All patients were instructed to otherwise maintain their usual GFD.

Small Bowel Mucosal Morphology and Immunohistochemistry

The efficacy end points were mucosal morphologic changes from baseline to post treatment in villus height to crypt depth ratio (VH:CrD), measures of small bowel mucosal inflammation (intraepithelial lymphocytes [IEL] density, autoreactive IgA deposits on duodenal mucosal TG2), and measures of serologic markers (TG2-IgA antibodies, endomysial IgA antibodies [EMA] and deamidated gliadin peptide [DGP] IgG and IgA antibodies).

At UGE, 4−7 small-bowel biopsy specimens were obtained from the descending duodenum; the specimens were read and evaluated by one investigator (KK) who was blinded to order of sampling. Three properly oriented biopsies were processed and stained with H&E for light microscopy. Morphometric analysis measuring VH:CrD was made separately from each biopsy specimen as described previously.[20−23] Only biopsies where the plane of sectioning was perpendicular to the luminal surface (ie, crypts cut longitudinally) were considered evaluable.[24] The mean value of 3 biopsies was reported; a mean ratio <2.0 was regarded as compatible with villus atrophy and crypt hyperplasia and indicative of active celiac disease. Patients with baseline VH:CrD values <2.0 were excluded from analysis. During the gluten challenge, a decrease in mean VH:CrD of ≥0.4 was considered clinically significant and indicated clinical gluten reactivity.[23,25]

The remaining biopsies were snap-frozen for immunohistochemical staining. The specimens were embedded in optimal cutting temperature compound (OCT; Tissue-Tec, Miles Inc, Elkhart, IN), snap-frozen in liquid nitrogen, and stored at −70°C. Immunohistochemical studies were performed on 5-μm−thick frozen sections; IELs ($CD3^+$ T-cells and $\alpha\beta$ and $\gamma\delta$ subsets) were stained and densities expressed as cells/mm of epithelium.[12,26] The upper limit of normal reference value for $CD3^+$ IELs was 37 cells/mm, for $\alpha\beta$ cells 25 cells/mm and for $\gamma\delta$ cells 4.3 cells/mm. After the gluten challenge, an increase in IEL densities ≥30% above baseline values was considered clinically significant and indicative of gluten reactivity in celiac disease patients.[23,25]

The small bowel mucosal TG2-specific autoantibody deposits were studied by direct immunofluorescence methods in unfixed frozen biopsy sections and semi-quantitatively graded from 0 to 3+.[25,27] An increase in the intensity of IgA-deposit staining after gluten challenge was considered indicative of gluten reactivity.

Celiac Disease Serum Autoantibodies

Serum TG2-IgA antibodies were assessed by ELISA (Quanta Lite h-tTG-IgA; Inova Diagnostics, San Diego, CA); the threshold for positive values was 20 IU. Serum IgA EMA was determined by an indirect immunofluorescence method using human umbilical cord as substrate; a serum dilution of 1:5 was considered positive;[28] all positive sera were serially diluted up to 1:4000. Patients were screened for selective IgA deficiency; if IgA deficient, a combination of serum IgA and IgG anti-DGP antibodies (QUANTA Lite Celiac DPG Screen, Inova Diagnostics, San Diego, CA) were assessed by ELISA; values ≥20 IU were considered positive. Seroconversion from negative to positive was considered clinically significant.

Safety Assessments

Patient safety was monitored by recording nonserious and serious 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 gluten ingestion or to study treatment. Additional safety assessments included physical examination, vital signs (ie, blood pressure, heart rate, and temperature), electrocardiography and serum chemistries, hematology, and urinalysis.

Gastrointestinal Symptoms and Quality of Life

Clinical symptoms appearing during the gluten challenge were classified into 4 categories (eg, no symptoms, slight, moderate, or severe symptoms) by patient interview and recorded at each visit by the study physicians.

Four instruments were used in this study to assess symptom (GSRS) and quality of life (VAS, CDQ, and Short Form-36 version 2) outcomes in patients during the study period.

The GSRS instrument and scoring methodology has been described previously.[29,30] The overall GSRS score was calculated as the mean of the nonmissing subdimensions.

The VAS used a 100-mm line, where 0 mm represented "excellent health, no symptoms and signs of celiac disease" and 100 mm represented "poor health, very severe symptoms and signs of celiac disease." Patients marked their status on the VAS line and the position of the mark was measured in millimeters from the left end to yield a 2-digit score.

The CDQ used 4 scales (ie, gastrointestinal symptoms, emotional well being, social restrictions, and disease-related worries) with 7 items on each scale and a 7-point ordinal scale for scoring.[31,32]

The Short Form-36 version 2 questionnaire was used to assess health-related quality of life.[33,34] Items were divided into the following 8 subdimensions: physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, emotional problems, and mental health. Each health domain yielded a score for both physical and mental health along with a single health utility index.

Statistical Analysis

The sample-size calculation for the therapeutic intervention study was based on the results from the gluten dose optimization study (see Figure 2) and previous investigator experiences with gluten challenges.[22] A change from baseline mean VH:CrD was expected to occur in approximately 70% of the patients resulting in an anticipated mean VH:CrD change of approximately 0.8 (SD ~0.6). Comparing the change from baseline in the primary end point, the sample size was calculated (including an anticipated 20% dropout rate) to achieve ≥80% power at the 5% significance level.

Two-sample *t* tests and the nonparametric Wilcoxon-Mann-Whitney methods were used to evaluate treatment group differences, where appropriate. Changes were calculated as post-treatment value minus baseline value, such that a negative change reflected a decrease and a positive change signified an increase. No judgment ("better" or "worse") was associated with the sign of the change, only direction. The likelihood ratio test was the primary statistical analytical method used to analyze treatment group differences for categorical response data. All statistical analyses were performed using a 2-sided hypothesis test at the 5% level of significance.

Continuous data were summarized using descriptive statistics: n, mean, SD or SE, median, minimum, and maximum. SDs were used when the analysis of interest was the natural variability of the data; SEs were used when comparing 2 or more means.

Ethics

Both the gluten-dose optimization and therapeutic intervention study protocols were approved by the Ethics Committee at Tampere University Hospital and by Finnish Regulatory Authorities. The studies were registered on Clinicaltrials.gov: the gluten dose optimization stage (Identifier: NCT00959114) and the therapeutic intervention with ALV003 (Identifier NCT01255696). The therapeutic intervention study was conducted at 3 medical centers in Tampere, Kuopio, and Oulu, Finland. All patients gave written informed consent. All authors had access to the data, reviewed, and approved the final manuscript.

Results

Gluten-Dose Optimization

Before conducting the therapeutic intervention study, the optimal gluten-challenge dose was identified. Forty-seven patients were enrolled (Table 1) and assigned to 1 of 3 groups receiving a gluten challenge of 6.0 g (n = 17), 3.0 g (n = 15), or 1.5 g (n = 15) daily divided in 3 doses. The baseline mean VH:CrD values were similar in each group: 2.8, 2.6, and 2.8, respectively. All patients in the 6 g gluten/day group completed the study; 2 patients in the 3.0 g/day and 1 in the 1.5 g/day gluten groups withdrew from the study before completing all the study visits because of acute onset of nausea, vomiting, or abdominal distension occurring <1 week into the gluten challenge. No follow-up biopsies were obtained from patients in whom the gluten challenge was <1 week. One patient each in the 1.5 g/day and 6.0 g/day groups were excluded from post-baseline evaluations due to extensive baseline mucosal inflammation (VH:CrD = 1.3 and 1.4, respectively). Therefore, the evaluable study group sizes were 16, 13, and 13 (6.0 g, 3.0 g, and 1.5 g groups, respectively).

With the 6-week gluten challenge, a clear gluten dose-response effect was observed (Figure 2). The mean post-treatment VH:CrD values were 1.1 (range, 0.2−2.7), 1.5

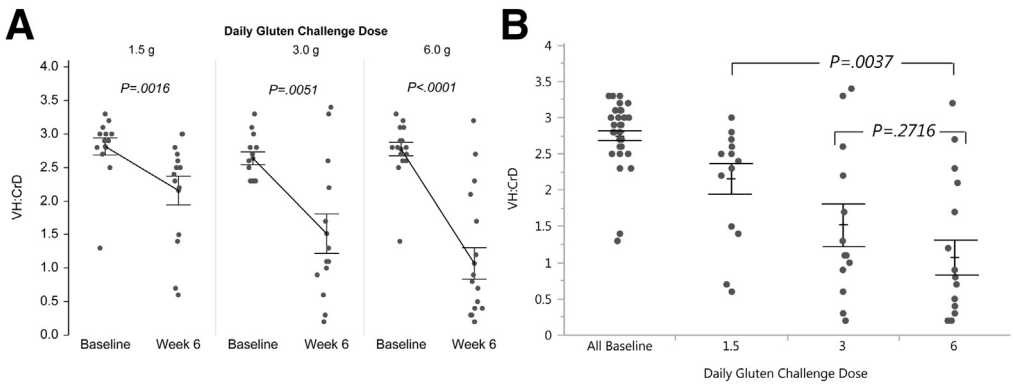

Figure 2. Gluten-dose optimization. (*A*) Mean (±SE) VH:CrD at baseline and end of the 6-week gluten challenge in patients on long-term GFD. (*B*) Six-week gluten challenge dose−response effect trend on VH:CrD (mean ± SE).

Table 1. Demographic Data of the Patients in the Gluten-Dose Optimization Study

Demographics	Gluten dose		
	6.0 g	3.0 g	1.5 g
n	17	15	15
Female, n (%)	13 (76)	7(47)	14 (93)
Age, *y*, median (range)	59 (42−70)	52 (42−67)	55 (41−74)
Duration of gluten free diet, *mo*, median (range)	130.4 (19−424)	104.5 (17−278)	131.0 (22−519)

(range, 0.2−3.3), and 2.2 (range, 0.6−3.0) for the 6-g, 3-g, and 1.5-g groups, respectively. The difference in VH:CrD measurements between the 6-g and 1.5-g groups was statistically significant ($P = .0037$) and there was a trend between 6-g and 3-g gluten dose, although the difference was not statistically significant ($P = .2716$). Results also showed that 1.5 g gluten given daily resulted in significant mucosal deterioration, while still appearing to be symptomatically well tolerated.

The change from baseline in VH:CrD after the 1.5-g challenge was not sufficiently consistent and too close to the baseline readout (ie, a "weak" mucosal injury signal). Additionally, we were concerned that a 3-g gluten challenge might not be well tolerated clinically, resulting in too many early dropouts. Therefore, for the therapeutic intervention study, 2 g gluten was chosen to be administered as a single daily dose.

Therapeutic Intervention

Forty-one patients were enrolled in the therapeutic intervention study between January and March 2011 and were administered the study treatment and a 6-week daily 2-g gluten challenge; all patients were followed for 4 weeks post treatment for safety assessments. Twenty and 21 patients were randomized to the ALV003 and placebo groups, respectively (Figure 3). The mean age was 53 years (range, 19−71 years) and 31 patients were women; patient demographics are shown in Table 2. Four patients (3 from the placebo-drug group) withdrew from the study early due to intolerable abdominal symptoms, including abdominal pain, abdominal distension, eructation and diarrhea; of these 4 patients, 3 (2 from the placebo group and 1 from the ALV003 group) withdrew during the first week and no post-treatment endoscopy was performed. The fourth patient, also from the placebo group, withdrew on day 18 and underwent post-treatment endoscopy with duodenal biopsy. Altogether, 19 patients in the ALV003 and 19 patients in the placebo groups received more than 1 week of study treatment and comprised the safety assessment group. However, 3 patients in the ALV003-treatment group and 1 patient in the placebo-treatment group were TG2-IgA−positive (by ELISA) at baseline, and were therefore excluded from the final efficacy analyses. The efficacy analyses (per protocol) were based on 16 patients in the ALV003 group and 18 patients in the placebo group.

Villus Height to Crypt Depth Ratio

At baseline, mean VH:CrD was 2.8 in both the ALV003- and placebo-treatment groups. After 6 weeks of daily gluten

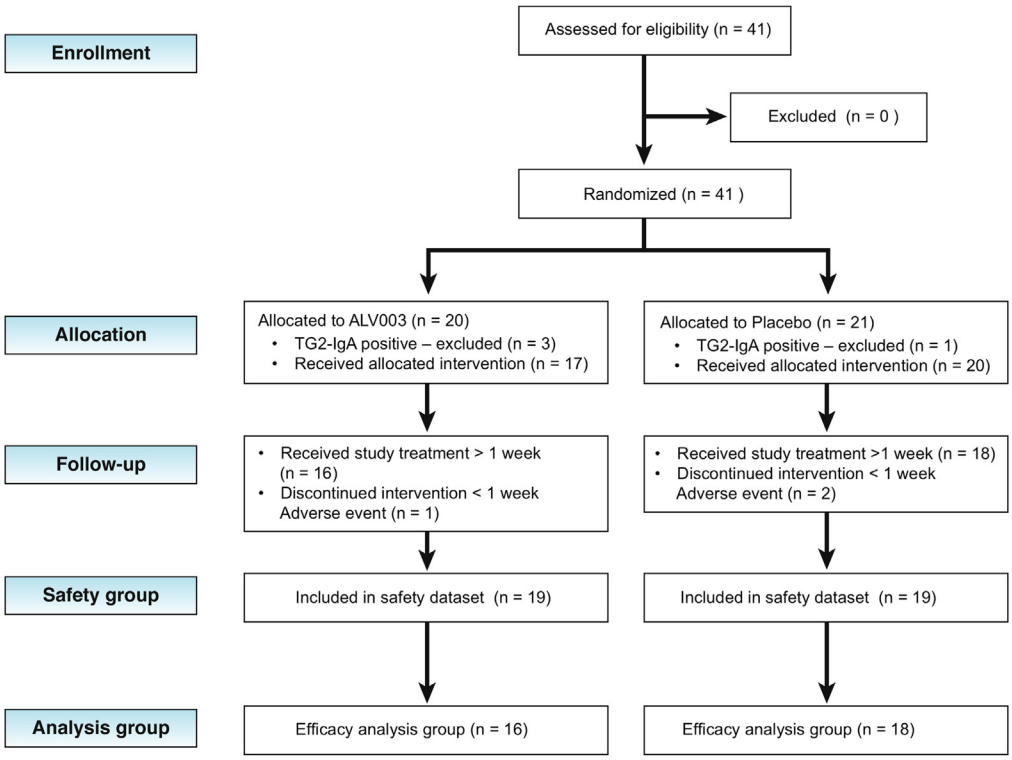

Figure 3. Distribution of patients in the therapeutic intervention study. ITT, intention to treat; TG2-IgA, transglutaminase 2 IgA class antibodies.

Table 2. Demographic Data of the Patients in the Therapeutic-Intervention Study

	ALV003	Placebo
n	20	21
Female, n (%)	14 (70)	17 (81)
Age, *y*, median (range)	58 (25–65)	50 (19–71)
Duration of gluten-free diet, *mo*, median (range)	135.5 (35–405)	147.0 (14–471)

exposure, the post-treatment biopsies demonstrated a statistically significant decrease in the median VH:CrD to 2.0 in the placebo drug group; $P = .0007$). In contrast, as shown in Figure 4*A*, thirteen of 16 patients in the ALV003-treatment group showed no significant mucosal deterioration (VH:CrD = 2.7; $P = .2499$). When comparing treatment groups, the ALV003 treatment group VH:CrD values remained significantly higher ($P = .0133$).

Intraepithelial Lymphocytes

At baseline, the mean $CD3^+$ T-cell densities (cells/mm) in the ALV003 and the placebo groups were similar (57 and 61, respectively; $P = .4271$). Post-treatment $CD3^+$ T-cell densities in the placebo-treated group were significantly elevated compared with baseline ($P = .0002$); however, the change in mean $CD3^+$ T-cell densities in the ALV003 group was not statistically different from baseline ($P = .7912$). When comparing the change in post-treatment IEL densities between treatment groups, the increase in $CD3^+$ T cells/mm in the placebo-treated patients compared with the ALV003-treated patients was statistically significant ($P = .0152$) (Figure 4*B*).

In the placebo-treated patients, the post-treatment $\alpha\beta$ T-cell densities (Figure 4*C*) increased from baseline levels (mean change, 24 cells/mm; range, −18 to 78; $P = .0010$), and there was no change from baseline to post-treatment densities for ALV003-treated patients (median change, −1.0 cells/mm; range, −60 to 28; $P = .7380$). Similar changes in the placebo and drug groups were observed in the $\gamma\delta$ T-cell densities (Figure 4*D*); between groups comparisons for both $\alpha\beta$ and $\gamma\delta$ T cells were statistically significant ($P = .0027$ and $P = .0030$, respectively).

Celiac Disease Serum Autoantibodies and Mucosal Deposits

At baseline, all the patients were negative for EMA antibodies. After 6 weeks of 2 g daily gluten exposure 1 patient in each the placebo- and ALV003-treatment groups seroconverted to positive EMA antibodies. Similarly, there were no statistically significant differences in TG2-IgA and DGP antibody titers from baseline between treatment groups.

The TG2-IgA deposits showed no statistically significant treatment differences from baseline or between groups (data not shown).

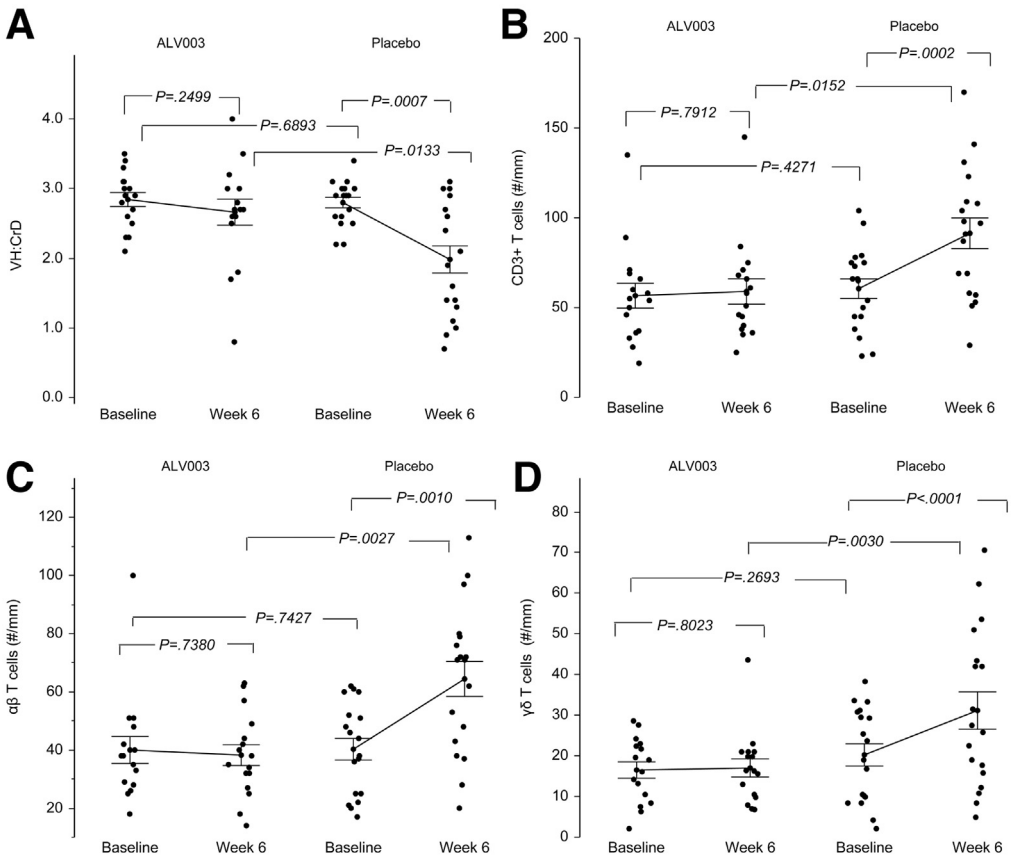

Figure 4. Duodenal mucosal biopsy morphometric data points (mean ± SE): (*A*) VH:CrD, (*B*) CD3+ IEL, (*C*) $\alpha\beta$ and (*D*) $\gamma\delta$ densities (cells/mm epithelium) in celiac disease patients on a long-term GFD at the baseline and after 6-week gluten challenge (2g/day).

Tolerability and Safety

ALV003 administered daily for up to 42 days appeared to be well tolerated in seronegative patients on a strict GFD undergoing a daily oral gluten challenge. The most frequent AEs leading to study discontinuation were moderate to severe nausea, vomiting, and abdominal pain, and were predominantly attributed to gluten exposure. No serious AEs occurred during this study. No clinically significant changes were observed in vital signs, safety laboratory tests, or electrocardiogram results.

Eighteen patients in the ALV003 group (90%) and 18 patients in the placebo group (85.7%) reported at least 1 treatment-emergent AE. The majority of the AEs were mild or moderate in severity and attributed to gluten ingestion. The most common AEs were gastrointestinal symptoms, reported by 75.6% of patients. The most common nongastrointestinal AE was headache, reported by 21.9% of patients (Table 3). Among patients who withdrew early from the study (1 ALV003 treated, 3 placebo; 1 placebo-treated patient underwent follow-up endoscopy and duodenal biopsy), none of the ALV003-treated patients experienced AEs that were reported to be severe, and 2 of the 3 placebo-treated patients who withdrew early from the study reported severe abdominal pain or diarrhea.

Symptom and Quality of Life Outcomes Measures

In the efficacy-analysis population there were consistent increases in overall GSRS scores from baseline through day 42, although by day 70 the scores returned to baseline. The change, during the challenge period, in overall GSRS, abdominal pain, and indigestion scores trended higher in the placebo patients (see details in Supplementary Table 2); all returned to baseline by day 70.

Table 3. Summary of Adverse Events

Adverse events	ALV003 (n = 20), n (%)	Placebo drug (n = 21), n (%)
Patients with any AE	18 (90.0)	18 (87.5)
Maximal severity		
Mild	11 (55.0)	9 (42.9)
Moderate	7 (35.0)	7 (33.3)
Severe	0 (0.0)	2 (9.5)
Most frequent AEs		
Gastrointestinal disorders	15 (75.0)	16 (76.2)
Abdominal distension	9 (45.0)	8 (38.1)
Flatulence	7 (35.0)	6 (28.6)
Eructation	3 (15.0)	4 (19.0)
Nausea	1 (5.0)	4 (19.0)
Diarrhea	3 (15.0)	3 (14.3)
Dyspepsia	2 (10.0)	2 (9.5)
Vomiting	1 (5.0)	3 (14.3)
Abdominal pain	1 (5.0)	2 (9.5)
Headache	5 (25.0)	4 (19.0)
Fatigue	1 (5.0)	6 (28.6)

The CDQ indicated that patients reported mild symptoms at baseline; by days 28 and 42 there were small deteriorations in overall quality of life and gastrointestinal scores in the placebo group, but in both treatment groups the scores recovered by day 70. Both the VAS and Short Form-36 version 2 failed to distinguish changes from baseline over time or between the study groups (data not shown).

Discussion

Many celiac disease patients desire a novel nondietary alternative to the strict life-long GFD.[14,15,35,36] Despite long-term dietary gluten exclusion, small intestinal mucosal injury with crypt hyperplasia (Marsh grade II–III) is still present in 20%–80% of patients.[12] In Finland, despite villus recovery in response to the GFD (96% with Marsh grade 0–I), persistent intraepithelial lymphocytosis is seen in more than half of patients studied.[12] Small bowel mucosal healing is a prerequisite for patients' long-term well being.[37–39]

There are 3 important findings in this report. The first is the observation that a mixture of 2 recombinant, orally administered gluten-specific proteases can attenuate gluten-induced mucosal injury in celiac disease patients as measured by change in villus-crypt morphometry and IEL densities. The second is demonstrating the efficiency of the gluten-challenge, using "real-world" gluten (in the form of baked breadcrumbs), to assess the safety and efficacy of a potential therapeutic agent. The third important finding is the value of using duodenal intestinal mucosal biopsies and the continuous measures of VH:CrD and IEL densities as end points to evaluate potential therapies for celiac disease.

In this randomized, double-blind, placebo-controlled clinical trial we show, for the first time, that a nondietary intervention, the gluten-specific protease ALV003, is able to attenuate gluten-induced injury to the small intestinal mucosa in celiac disease patients. Statistically and clinically significant differences in villus-crypt morphometric responses to daily gluten challenges, given in the context of everyday gluten-free meals, are observed between the treatment and placebo groups. The primary observation, maintenance of the mucosal architecture by ALV003, is strengthened by the finding that mucosal inflammatory responses (ie, change in IEL densities) are obviated. These results are consistent with nonclinical and early clinical studies demonstrating that ALV003 could degrade gluten proteins into nonimmunogenic peptide fragments.[16,17] Time-course studies in celiac disease show that with lower-dose gluten challenges an inflammatory process characterized by the accumulation of IEL occurs; with further challenge, dose-dependent crypt hyperplasia initially appears, followed by villus effacement.[40] For any potential therapy to be considered clinically effective, it should significantly reduce or prevent gluten-induced mucosal injury.

The small intestinal mucosal biopsy for histologic evaluation remains the gold standard in measuring gluten-induced mucosal injury.[1] Because a low-dose gluten challenge given for a short period of time was used in this study,

a rigorous quantitative measure of mucosal injury in the proximal duodenum was used as the primary end point. The choice of a histologic end point distinguishes this trial from other recently completed gluten challenge drug trials.[41,42]

To evaluate whether any putative therapy is able to attenuate gluten-induced mucosal injury, reliable and reproducible duodenal histologic assessments that can detect small but significant changes are required. The grouped histologic classification of Marsh-Oberhuber, extensively used in celiac disease clinical diagnosis, has a high inter-observer variability.[43–45] We used morphometric techniques that measure the continuum of gluten-induced mucosal responses, specifically the VH:CrD and the IEL densities.[20,22,46,47] To minimize the variability inherent in interpreting mucosal morphometric parameters, we standardized histologic methodology in this study to ensure measurable villus-crypt units with the evaluable crypts cut longitudinally.[21–24] In fact, quantitative, reliable, and reproducible morphometric results (VH:CrD and IEL densities) can be obtained on duodenal biopsy specimens with different grades of gluten-induced injury.[23]

The characteristics of the dietary gluten-induced "dose-response" to the mucosal injury have not previously been rigorously defined. We performed our gluten challenges using baked breadcrumbs rather than Frazer's fraction III gluten digests, as used by others.[40] Our gluten-challenge dose-identification study defined an operative range of gluten challenge doses to be used in a therapeutic intervention trial. In contrast to the 2-week data by Leffler et al,[47] we demonstrated a dose-dependent effect of gluten on the mucosal morphology during a 6-week challenge period (Figure 2).

The sample size calculation for this study was based on the expected change in VH:CrD from baseline to week 6. However, the small sample size did not allow demonstration of statistically significant differences in either serum TG2-IgA, DGP antibodies, or in symptoms.

Daily oral administration of ALV003 appeared to be well tolerated in the celiac disease patients who were under good clinical control while on a GFD and challenged with 2 g dietary gluten daily for 6 weeks (equivalent to approximately one half of standard slice of bread in the United States). The majority of AEs reported were consistent with those typically seen in celiac disease patients ingesting gluten. Using a nonceliac disease-specific symptom instrument (GSRS) limited the ability to detect changes in disease-specific symptoms. We were unable to quantify a possible "nocebo" effect because of the lack of a gluten-placebo arm, which could also account for the lack of major differences in reported symptoms between treatment arms, as patients might have anticipated onset of gastrointestinal symptoms after deliberate ingestion of gluten.[48,49] There were no serious AEs or deaths in the study.

In conclusion, for the first time, a pharmaceutical agent targeting degradation of immunogenic gluten peptides has the ability to attenuate gluten-induced injury to the small intestinal mucosa in celiac disease patients. Accordingly, the results support the hypothesis that targeting proline- and glutamine-rich gluten peptides is a potentially viable approach for the treatment of celiac disease. Evaluation of ALV003 in patients with mucosal inflammation is warranted.

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.2014.02.031.

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Conflicts of interest
These authors disclose the following: Marja-Leena Lähdeaho is the principal investigator and is employed by FinnMedi Oy. Tiina Kärjä-Lahdensuu is an employee of FinnMedi Oy. Annette Marcantonio and Daniel C. Adelman are employees of Alvine Pharmaceuticals, Inc. Markku Mäki is a scientific advisor to Alvine Pharmaceuticals, Inc., ImmusanT, Inc, BioLineRx, Ltd, and Flamentera, AG. The remaining authors disclose no conflicts.

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