ORIGINAL ARTICLE

A Randomized Trial of a Transglutaminase 2 Inhibitor for Celiac Disease

D. Schuppan, M. Mäki, K.E.A. Lundin, J. Isola, T. Friesing-Sosnik, J. Taavela, A. Popp, J. Koskenpato, J. Langhorst, Ø. Hovde, M.-L. Lähdeaho, S. Fusco, M. Schumann, H.P. Török, J. Kupcinskas, Y. Zopf, A.W. Lohse, M. Scheinin, K. Kull, L. Biedermann, V. Byrnes, A. Stallmach, J. Jahnsen, J. Zeitz, R. Mohrbacher, and R. Greinwald, for the CEC-3 Trial Group*

ABSTRACT

BACKGROUND

In celiac disease, small intestinal transglutaminase 2 causes deamidation of glutamine residues in gluten peptides, which enhances stimulation of T cells and leads to mucosal injury. Inhibition of transglutaminase 2 is a potential treatment for celiac disease.

METHODS

In a proof-of-concept trial, we assessed the efficacy and safety of a 6-week treatment with ZED1227, a selective oral transglutaminase 2 inhibitor, at three dose levels as compared with placebo, in adults with well-controlled celiac disease who underwent a daily gluten challenge. The primary end point was the attenuation of gluten-induced mucosal damage, as measured by the ratio of villus height to crypt depth. Secondary end points included intraepithelial lymphocyte density, the Celiac Symptom Index score, and the Celiac Disease Questionnaire score (for assessment of health-related quality of life).

RESULTS

Of the 41 patients assigned to the 10-mg ZED1227 group, the 41 assigned to the 50-mg group, the 41 assigned to the 100-mg group, and the 40 assigned to the placebo group, 35, 39, 38, and 30 patients, respectively, had adequate duodenal-biopsy samples for the assessment of the primary end point. Treatment with ZED1227 at all three dose levels attenuated gluten-induced duodenal mucosal injury. The estimated difference from placebo in the change in the mean ratio of villus height to crypt depth from baseline to week 6 was 0.44 (95% confidence interval [CI], 0.15 to 0.73) in the 10-mg group ($P=0.001$), 0.49 (95% CI, 0.20 to 0.77) in the 50-mg group ($P<0.001$), and 0.48 (95% CI, 0.20 to 0.77) in the 100-mg group ($P<0.001$). The estimated differences from placebo in the change in intraepithelial lymphocyte density were –2.7 cells per 100 epithelial cells (95% CI, –7.6 to 2.2) in the 10-mg group, –4.2 cells per 100 epithelial cells (95% CI, –8.9 to 0.6) in the 50-mg group, and –9.6 cells per 100 epithelial cells (95% CI, –14.4 to –4.8) in the 100-mg group. Use of the 100-mg dose may have improved symptom and quality-of-life scores. The most common adverse events, the incidences of which were similar across all groups, were headache, nausea, diarrhea, vomiting, and abdominal pain. Rash developed in 3 of 40 patients (8%) in the 100-mg group.

CONCLUSIONS

In this preliminary trial, treatment with ZED1227 attenuated gluten-induced duodenal mucosal damage in patients with celiac disease. (Funded by Dr. Falk Pharma; CEC-3 EudraCT number, 2017-002241-30.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Schuppan at the Institute of Translational Immunology and Celiac Center, Research Center for Immune Therapy, University Medical Center, Johannes Gutenberg University, Langenbeckstr. 1, 55131 Mainz, Germany, or at detlef.schuppan@unimedizin-mainz.de.

*A complete list of the investigators and contributors in the CEC-3 Trial Group is provided in the Supplementary Appendix, available at NEJM.org.

Drs. Schuppan and Mäki contributed equally to this article.

N Engl J Med 2021;385:35-45.
DOI: 10.1056/NEJMoa2032441

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Celiac disease is characterized by inflammation of the small intestine, is frequently associated with autoimmunity, and affects 0.2 to 2.0% of the population in most countries.[1-3] The identification of cases has increased in the past decades owing to improved serologic diagnosis, as has the true prevalence of celiac disease.[1,4] Celiac disease is driven by the ingestion of gluten in wheat and related grains in genetically predisposed persons who have HLA-DQ2 and HLA-DQ8 genotypes, which are necessary but not sufficient for the manifestation of celiac disease. The classic symptoms of celiac disease are diarrhea, weight loss, and malnutrition, but celiac disease frequently manifests with nonspecific or atypical symptoms, including fatigue, altered bowel habits, anemia, osteoporosis, or autoimmune diseases such as autoimmune thyroiditis and type 1 diabetes.[5-10]

Active celiac disease is diagnosed on the basis of elevated levels of serum autoantibodies to transglutaminase 2 and is confirmed by histologic villus atrophy and crypt hyperplasia in the proximal small intestine, accompanied by intraepithelial lymphocyte infiltration in duodenal mucosa.[11] The only available treatment for celiac disease is lifelong adherence to a strict gluten-free diet, a diet that is difficult to maintain,[12] and only 50% of patients have mucosal recovery and often do not have negative serum autoantibodies 1 year or later after diagnosis.[13] Moreover, many patients with celiac disease report having persistent symptoms despite adherence to the gluten-free diet.[14] Thus, there is an unmet medical need for an effective treatment adjunct to a strict gluten-free diet. Currently, no drug therapy reliably prevents the effects of dietary gluten or has been approved by regulators to treat celiac disease.

Transglutaminase 2, the celiac autoantigen,[8,15-17] is expressed in the intestinal mucosa, where it modifies immunogenic gluten peptides by means of deamidation of certain charge-neutral glutamine residues, yielding negatively charged glutamic acid residues.[15,17] This modification promotes gluten-peptide presentation by HLA-DQ2 or HLA-DQ8 molecules on mucosal antigen–presenting cells[8,10] and enables the activation and expansion of gluten peptide–specific CD4+ type 1 helper T cells and the secretion of proinflammatory cytokines. This process leads to villus atrophy and crypt hyperplasia and to B-cell differentiation and the production of transglutaminase 2 IgA.[8,10,15-17]

ZED1227 inhibits transglutaminase 2 with high specificity and prevents the formation of deamidated gluten and, putatively, the initial steps of gluten-induced T-cell activation.[18] ZED1227 is formulated as an oral capsule for duodenal targeting and has been tested for clinical safety in earlier studies (EudraCT numbers, 2014-003044-13 and 2015-005283-42 [data on file]). Our phase 1 clinical studies, which involved 106 healthy persons who were exposed to doses of up to 500 mg of ZED1227 for up to 8 days, did not show drug-related adverse effects or signs of drug toxicity, and systemic drug levels after oral ingestion were low. Here, we report the results of a phase 2, randomized, double-blind, placebo-controlled, dose-finding trial of ZED1227 capsules to evaluate efficacy and safety in adult patients with celiac disease in histologic and serologic (transglutaminase 2 IgA) remission owing to a gluten-free diet who were challenged with daily gluten intake for 6 weeks.

METHODS

TRIAL OVERSIGHT

We conducted this trial at 20 sites in seven countries (Estonia, Finland, Germany, Ireland, Lithuania, Norway, and Switzerland). The trial was approved by an independent ethics committee at each site. Written informed consent was obtained from each patient before screening. The sponsor, Dr. Falk Pharma, contributed to the trial design, data analysis, and the writing and editing of the manuscript. Data were collected by the investigators with the use of electronic case-report forms. All the authors vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol, which is available with the full text of this article at NEJM.org.

TRIAL PATIENTS

Adults 18 to 65 years of age who had received a biopsy-confirmed diagnosis of celiac disease at least 12 months before screening and who were positive for HLA-DQ2 or HLA-DQ8 genotypes were considered for inclusion in the trial. Patients had to have successfully adhered to a gluten-free diet for at least 12 months, to present with negative serologic testing for transglutaminase 2

IgA and a mean ratio of villus height to crypt depth of 1.5 or higher, and to agree to tolerate a challenge of ingesting 3 g of gluten daily for 6 weeks. Full inclusion and exclusion criteria are provided in the Supplementary Appendix, available at NEJM.org.

TRIAL DESIGN AND TREATMENT

In this double-blind, placebo-controlled, dose-ranging trial, eligible patients were randomly assigned, in a 1:1:1:1 ratio, to one of four parallel groups to receive 10 mg of ZED1227, 50 mg of ZED1227, 100 mg of ZED1227, or placebo, all with matched appearance, concurrent with the gluten challenge (Fig. S1 in the Supplementary Appendix). Each morning after at least 6 hours of fasting, patients took ZED1227 or placebo orally and, 30 minutes later, ate one sponsor-provided biscuit containing 3 g of gluten before breakfast. Throughout the 6-week trial, patients were required to continue their strict gluten-free diet, aside from eating the sponsor-provided gluten-containing biscuit.

Duodenal mucosal damage as an objective marker of gluten-induced celiac disease activity can be measured quantitatively by means of standardized histopathological morphometry.[19] The gluten challenge, in which a moderate amount of gluten is ingested daily for a limited duration, produces a statistically and clinically significant but reversible deterioration of duodenal mucosa and allows for the assessment of efficacy of the active treatment.[20-23]

END POINTS

The primary end point was the attenuation of gluten-induced deterioration of intestinal mucosal morphologic features, as measured by the ratio of villus height to crypt depth in duodenal-biopsy samples obtained starting at the baseline (screening) visit to the end of the 6-week gluten challenge and treatment period.[24] Secondary end points included changes from baseline to week 6 in the density of CD3+ intraepithelial lymphocytes, the modified Marsh–Oberhuber classification,[11] patient-reported outcomes as measured by the Celiac Symptom Index[25] and the Celiac Disease Questionnaire,[26] blood markers of malabsorption (e.g., ferritin, transferrin saturation, and albumin), plasma concentrations of ZED1227, and serologic markers of celiac disease. On the modified Marsh–Oberhuber classification, a score of 0 indicates normal duodenal morphology without increased intraepithelial lymphocytes; a score of 1, normal duodenal morphology with increased intraepithelial lymphocytes; a score of 2, normal villi but crypt hyperplasia; and scores of 3a through 3c, increasing severity of villus atrophy and crypt hyperplasia, with a score of 3c indicating complete villus atrophy. The Celiac Symptom Index is a 16-item questionnaire, with each item rated on a scale of 1 (no symptoms) to 5 (symptoms all the time); overall scores range from 16 to 80, with higher scores indicating worse celiac disease–related symptoms.[25] The Celiac Disease Questionnaire is a 28-item questionnaire, with each item rated from 1 (reduced health-related quality of life) to 7 (high health-related quality of life); overall scores range from 28 to 196, with higher scores indicating better quality of life.[26]

Safety was evaluated by the monitoring of adverse events, vital signs, body weight, clinical laboratory tests, and side-effect profile as assessed by the investigator and the patient. For the assessment of change in the ratio of villus height to crypt depth, patients were excluded from the analysis if they did not have adequate follow-up duodenal-biopsy samples that allowed for the measurement of at least three separate villus–crypt units.[24] A sensitivity analysis included all the patients who underwent randomization and received at least one dose of ZED1227 or placebo. All the other efficacy analyses involved patients who underwent randomization and received at least one dose of ZED1227 or placebo, without imputation of missing values at follow-up; modeling was performed with the use of complete cases only.

TRIAL PROCEDURES AND ASSESSMENTS

Enrollment required a screening period of no more than 8 weeks, including upper gastrointestinal endoscopy with duodenal biopsies performed within 4 weeks before the administration of the first dose in order to provide baseline histologic data. At the week 0 visit, the trial drug (ZED1227 or placebo) and gluten biscuits were dispensed to patients according to assigned group. Patients returned to the trial sites at weeks 2, 4, and 6 for assessments and at week 10 for a follow-up visit. A second endoscopy with biopsies was performed at the week 6 or withdrawal visit. Both endoscopies were conducted by experienced gastroenter-

ologists. Four forceps biopsy samples (one biopsy sample per pass) were obtained from the second and third parts of the duodenum, put in a PAXgene fixative, and sent to the central histopathology laboratory (Jilab, Tampere, Finland) for processing and reading. Validated, standardized morphometric procedures separately evaluated mucosal morphology and inflammation.[24,27] The categorical Marsh–Oberhuber classification[11] was used for the standard classification of the mucosal lesions (see the Supplementary Appendix).

Patients kept a diary (on paper or electronically) to record their daily use of the assigned trial drug as well as their gluten biscuit and food intake, concomitant medications, and stool frequency and characteristics. Scores on the Celiac Symptom Index and Celiac Disease Questionnaire were determined at all visits. At the end of the treatment period (week 6), investigators and patients independently rated the trial treatment as being "very good," "good," "satisfactory," or "poor" with regard to both efficacy and the side-effect profile.

Adverse events were recorded and evaluated by the investigators. Blood samples were obtained to determine hematologic, coagulation, and serum markers.

STATISTICAL ANALYSIS

We estimated that a sample of 136 patients, or 34 patients per group, would provide the trial with 80% power for the primary analysis, assuming an alpha error of 0.05, an effect size of 0.6, and standard deviation of 0.8. On the basis of an estimated 15% of patients either withdrawing or having insufficient samples for evaluation, we planned for the enrollment of 160 patients (40 per group).

The primary end point, the mean change in the ratio of villus height to crypt depth from baseline to week 6, was analyzed with the use of a generalized linear model with the identity link, in which trial group and the baseline ratio of villus height to crypt depth were fixed effects, because assumptions for the parametric model were met. Each ZED1227 dose group was compared with the placebo group. The same statistical method was used for the analyses of the change from baseline to week 6 in the intraepithelial lymphocyte density, Celiac Symptom Index scores, and Celiac Disease Questionnaire scores, which were key secondary end points. The statistical analysis of other end points (the change of Marsh–Oberhuber classification, transglutaminase 2 IgA and IgA antiendomysial antibodies, and blood malabsorption markers) is described in the Supplementary Appendix. The analysis to assess sensitivity to missing data is described in the Supplementary Appendix.

Plasma concentrations of ZED1227 and metabolites were measured and analyzed for pharmacokinetic profiles. The results are not reported here.

All statistical comparisons between each ZED1227 dose and placebo were two-sided, with a familywise alpha error of 0.05. For the analysis of the primary end point, 95% confidence intervals and P values were adjusted for multiple comparisons with the use of Bonferroni correction to account for the three comparisons with placebo. An adjusted P value of 0.0167 was considered to indicate statistical significance for the primary end point, and individual confidence intervals were constructed with the use of 98.3% levels. For secondary end points, 95% confidence intervals are reported without P values; the 95% confidence intervals have not been adjusted for multiple comparisons and cannot be used to infer definitive treatment effects. One interim analysis was conducted for the primary efficacy variable (see the Supplementary Appendix).

RESULTS

CHARACTERISTICS OF THE PATIENTS AND REGIMEN ADHERENCE

The trial was conducted from May 16, 2018, to February 27, 2020. Of the 163 patients who underwent randomization (with 41 patients assigned to the 10-mg ZED1227 group, 41 to the 50-mg group, 41 to the 100-mg group, and 40 to the placebo group), 3 did not receive ZED1227 or placebo because of the development of other clinical conditions, and 1 patient received ZED1227 but was lost to follow-up (Fig. S2). Analyses of efficacy excluded these 4 patients and thus included 41 patients in the 10-mg group, 41 in the 50-mg group, 39 in the 100-mg group, and 38 in the placebo group.

The demographic characteristics of these 159 patients were similar across the groups, except for a higher percentage of women in the 10-mg group (Table 1). According to the investigators' assessment and patients' diaries, adherence was

Table 1. Demographic Characteristics of the Patients.*

Characteristic	ZED1227, 10 mg (N=41)	ZED1227, 50 mg (N=41)	ZED1227, 100 mg (N=39)	Placebo (N=38)
Age — yr	40.2±12.4	42.8±12.1	41.0±14.8	42.5±14.4
Female sex — no. (%)	37 (90)	29 (71)	24 (62)	28 (74)
White race — no. (%)†	41 (100)	41 (100)	39 (100)	38 (100)
Weight — kg	70.9±12.9	71.8±13.1	73.2±13.7	68.4±14.7

* Plus–minus values are means ±SD. Shown are data for 159 of the 163 patients who underwent randomization. Three patients who did not receive the assigned trial drug (ZED1227 or placebo) and 1 patient who was lost to follow-up were excluded from the efficacy analyses, so their demographic characteristics are not shown here.
† Race was determined by the investigator.

Table 2. Effect of ZED1227 Treatment on the Ratio of Villus Height to Crypt Depth.*

Variable	ZED1227, 10 mg (N=35)	ZED1227, 50 mg (N=39)	ZED1227, 100 mg (N=38)	Placebo (N=30)
Ratio of villus height to crypt depth				
At baseline	2.01±0.30	2.04±0.32	2.09±0.35	1.98±0.33
After gluten challenge at wk 6	1.85±0.53	1.91±0.44	1.94±0.48	1.39±0.61
Change in ratio from baseline (95% CI)†	−0.17 (−0.33 to −0.01)	−0.12 (−0.27 to 0.03)	−0.13 (−0.28 to 0.03)	−0.61 (−0.78 to −0.44)
Estimated difference in ratio vs. placebo (95% CI)‡	0.44 (0.15 to 0.73)	0.49 (0.20 to 0.77)	0.48 (0.20 to 0.77)	—
P value	0.001	<0.001	<0.001	—

* Plus–minus values are means ±SD. As stipulated in the trial protocol, the primary end-point analysis included all the patients who underwent randomization and had villus height and crypt depth measurements from at least three separate villus–crypt units of sufficient quality in total from the duodenum biopsies available at both the baseline (screening) visit and the final or withdrawal visit (142 patients). A total of 16 patients (6 in the 10-mg group, 2 in the 50-mg group, 1 in the 100-mg group, and 7 in the placebo group) were excluded because they did not undergo final endoscopy; in addition, 1 patient in the placebo group was excluded because one of the samples obtained was not adequate for analysis.
† The change from baseline is presented as a least-squares means estimate.
‡ For the estimate of the treatment difference, Bonferroni correction was used for adjustment of the 95% confidence intervals and P values. The adjusted P value that was required in order to declare significance for the primary end point was 0.0167, and individual confidence intervals were constructed with the use of 98.3% levels.

high and similar in all four groups, ranging from 96 to 100% both for the trial regimen and for gluten intake.

EFFICACY RESULTS

Histology-related efficacy end points could be evaluated in 142 patients who had sufficient biopsy samples at both baseline and week 6; a total of 35 patients in the 10-mg group, 39 in the 50-mg group, 38 in the 100-mg group, and 30 in the placebo group were included in the analysis. Four of these patients stopped treatment before week 6 but qualified for inclusion in the primary end-point analysis with a treatment duration of 23 to 32 days.

As expected, in the placebo group, the gluten challenge decreased the ratio of villus height to crypt depth from baseline to week 6 (estimated change, −0.61; 95% confidence interval [CI], −0.78 to −0.44). Treatment with daily doses of 50 mg and 100 mg of ZED1227 prevented this deterioration (estimated change, −0.12 [95% CI, −0.27 to 0.03] and −0.13 [95% CI, −0.28 to 0.03], respectively); efficacy in the 10-mg group was slightly less (estimated change, −0.17; 95% CI, −0.33 to −0.01). As compared with placebo, all three dose levels of ZED1227 significantly prevented a decrease in the ratio of villus height to crypt depth (P≤0.001 for all comparisons) (Table 2 and Figs. 1 and S3A). Results of the sensitivity analysis were similar to those of the primary end-point analysis (Table S1).

Gluten ingestion caused an increase from baseline in intraepithelial lymphocyte density, a

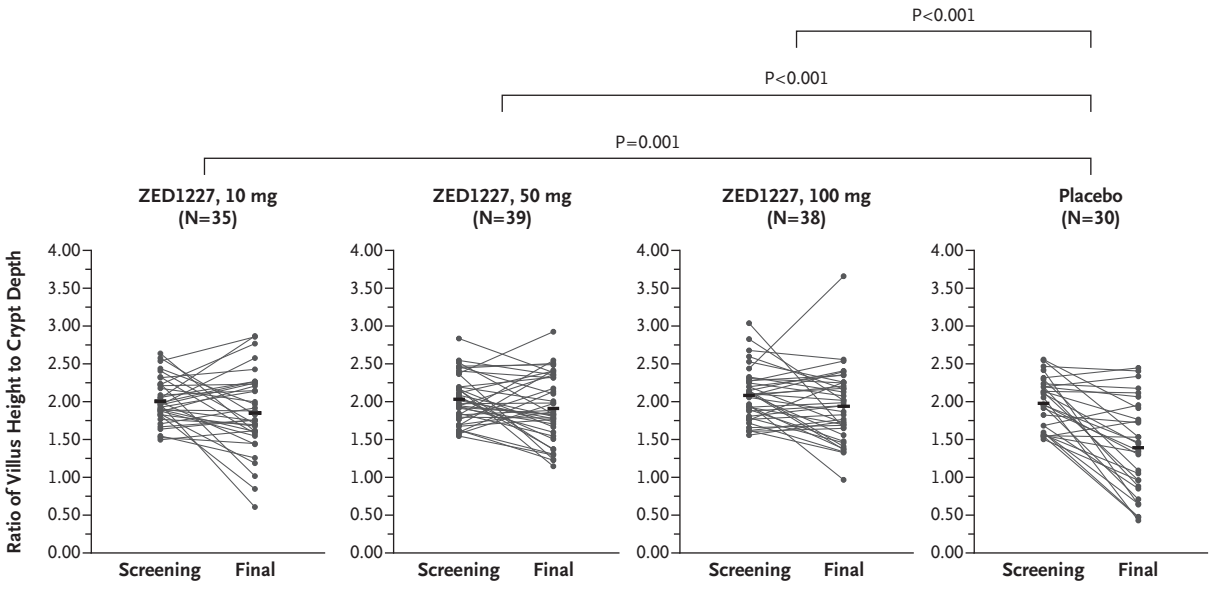

Figure 1. Paired Data Plots of the Mean Ratio of Villus Height to Crypt Depth (Primary End Point).

The primary end point was the attenuation of gluten-induced mucosal damage, as measured by the ratio of villus height to crypt depth. The prespecified population for this analysis included the 142 patients who had villus height and crypt depth measurements from at least three villus–crypt units in total from the duodenum biopsies available at both the screening (baseline) visit and the final or withdrawal visit. Horizontal bars indicate mean values in each group at each time point.

key variable of mucosal inflammation, in the placebo group, the 10-mg group, and the 50-mg group but not in the 100-mg group; the increase was attenuated by ZED1227 dose-dependently (Table 3 and Figs. S3B and S4). According to the modified Marsh–Oberhuber classification, a histologic score in which class 2 describes deepened crypts and class 3a to 3c categorically describes increasing severity of villus atrophy and increasing crypt depth, the odds and risk ratios favored all three doses of ZED1227 over placebo (Tables S2 and S3).

The Celiac Symptom Index score increased from baseline to week 6 in all groups and returned to baseline at the follow-up visit. Comparison with placebo favored all dose levels of ZED1227. The Celiac Disease Questionnaire overall score increased from baseline to week 6 in all ZED1227 dose groups but decreased with placebo. The changes in the Celiac Disease Questionnaire gastrointestinal subscore from baseline to week 6 and comparison with placebo favored ZED1227. At the week 6 visit, 10% of the patients in the 10-mg group, 7% of those in the 50-mg group, 8% of those in the 100-mg group, and 26% of those in the placebo group assessed the efficacy as "poor." Data are shown in Tables 3 and S4 and Figure S5.

At screening (baseline), all the patients had normal levels of transglutaminase 2 IgA. At the week 6 assessment, 1 patient (2%) who received the 10-mg dose, 1 (2%) who received the 50-mg dose, and no patient who received the 100-mg dose, as compared with 6 patients (16%) who received placebo, had a conversion to a positive result. The titer of transglutaminase 2 IgA increased by a mean of 2.4 kU per liter in the placebo group, by 1.7 kU per liter in the 10-mg group, and by 0.3 kU per liter in the 50-mg group and decreased by 0.1 kU per liter in the 100-mg group. Similar results were obtained regarding IgA antiendomysial antibodies (Tables S5 and S6).

SAFETY

Adverse events occurred in 125 of the 160 patients (78%) who received at least one dose of ZED1227 or placebo (Table 4). (In addition to the 159 patients in the efficacy analysis, 1 patient was added to the 100-mg group for the safety

analysis because this patient received the trial drug but was lost to follow-up, so the administration of the medication was uncertain.) Most adverse events appeared to be related to the gluten challenge. A total of 74 patients (46%), including 14 of 41 patients (34%) in the 10-mg group, 19 of 41 (46%) in the 50-mg group, 20 of 40 (50%) in the 100-mg group, and 21 of 38 (55%) in the placebo group, had an adverse event that the investigators considered to be potentially related to ZED1227 or placebo. The most common adverse events across all groups were headache, nausea, diarrhea, vomiting, and abdominal pain. With the exception of rash, which occurred in 3 patients (8%) in the 100-mg group, no adverse events appeared to be more common in the ZED1227 groups than in the placebo group. Serious adverse events that were considered by the investigators to be related to ZED1227 or placebo occurred in 2 patients (migraine with aura in 1 patient receiving 50 mg of ZED1227 and ventricular extrasystoles in 1 patient receiving placebo). These 2 patients discontinued ZED1227 or placebo and recovered.

A broad range of variables were measured in blood, including cell counts, liver and kidney functions, surrogates of resorption (albumin, transferrin saturation, zinc, vitamin B_{12}, and folic acid), and factor XIII, another common transglutaminase. Results were similar across all the trial groups. During the gluten challenge, the levels of alanine aminotransferase and alkaline phosphatase increased from baseline to week 6 in the placebo group and then normalized at week 10. Such changes were not observed in any of the ZED1227 groups (Table S7).

DISCUSSION

In this preliminary, randomized, double-blind, placebo-controlled, 6-week trial, the effectiveness of transglutaminase 2 inhibition with the oral transglutaminase 2 inhibitor ZED1227 was shown in patients with celiac disease who were challenged with a moderate amount (3 g) of daily gluten intake. ZED1227 was developed to specifically block transglutaminase 2–mediated potentiation of gluten-peptide immunogenicity in the small intestinal mucosa, a key driver of celiac disease pathogenesis.[8,9] As compared with placebo, all doses of ZED1227 attenuated the gluten-induced small intestinal damage.

This trial showed that treatment with the highly specific transglutaminase 2 inhibitor ZED1227 attenuated the gluten-induced damage in the duodenal mucosa. The ratio of villus height to crypt depth is widely considered to be the most objective and valid primary end point in clinical studies for therapies for celiac disease,[24,28-30] and the end point was achieved at all three dose levels of ZED1227. The benefits across multiple end points were most pronounced for the 50-mg and 100-mg doses. Improved patient-reported outcomes across the dose groups need to be confirmed in a larger study, since they may reflect the rather small size of each group for the evaluation of symptoms or the scales capturing some symptoms that may overlap with, but are unrelated to, celiac disease. Overall, the incidence and severity of adverse events were similar in the ZED1227 groups and the placebo group.

Since the discovery of transglutaminase 2 as the autoantigen in celiac disease,[15] extensive research has confirmed it to be a crucial mechanistic driver of gluten-induced inflammation and clinical manifestations in patients with celiac disease.[8,10,16,17] This trial supports the role of transglutaminase 2 in the pathogenesis of celiac disease, given that its inhibition prevents the deamidation of gluten peptides in the small intestinal mucosa and thus abolishes the immunogenic process. ZED1227 targets the intestinal mucosa predominantly and thereby mediates protection; thus, it is unaffected by the complexity of the food matrix and is less dependent on the timing of ingestion of gluten-containing food.

The Food and Drug Administration recently reinforced its recommendation that, in pharmacologic trials of celiac disease, the prevention of histologic damage should be a major end point in phase 2 clinical studies, and the improvement of celiac disease–related patient-reported outcomes and quality of life should both be primary end points in phase 3 trials.[30] This recommendation is justified, since only 40% of adult patients with newly diagnosed celiac disease have gastrointestinal symptoms, whereas a gluten challenge induces a manifest duodenal mucosal lesion, often before any symptoms occur.[22,31] Furthermore, mucosal healing is considered to be the key criterion of successful treatment and a prerequisite for patients' long-term well-being and the prevention of severe complications.[32-34] Therefore, we selected the gluten challenge and used vali-

Table 3. Effect of ZED1227 Treatment on Intraepithelial Lymphocyte Density and Scores on the Celiac Symptom Index and Celiac Disease Questionnaire.*

Variable	ZED1227, 10 mg	ZED1227, 50 mg	ZED1227, 100 mg	Placebo
Intraepithelial lymphocyte density				
Baseline				
No. of patients	41	41	39	38
No. of cells per 100 epithelial cells	26.5±6.8	29.3±9.0	26.4±8.4	27.9±10.2
After gluten challenge at wk 6				
No. of patients	35	39	38	31
No. of cells per 100 epithelial cells	34.6±12.0	35.7±12.0	27.9±7.8	38.6±15.7
Change from baseline (95% CI) — no. of cells per 100 epithelial cells†	8.3 (5.0 to 11.7)	6.9 (3.7 to 10.1)	1.5 (–1.8 to 4.7)	11.0 (7.4 to 14.6)
Estimated difference vs. placebo (95% CI) — no. of cells per 100 epithelial cells	–2.7 (–7.6 to 2.2)	–4.2 (–8.9 to 0.6)	–9.6 (–14.4 to –4.8)	—
Celiac Symptom Index‡				
Baseline				
No. of patients	38	39	38	33
Score	24.4±5.6	27.0±7.8	24.2±5.1	26.0±5.8
After gluten challenge at wk 6				
No. of patients	38	38	37	32
Score	25.9±6.1	29.0±8.0	25.2±5.8	29.8±9.4
Change from baseline (95% CI)†	0.9 (–1.0 to 2.8)	2.0 (0.0 to 3.9)	0.1 (–1.8 to 2.1)	4.0 (1.8 to 6.1)
Estimated difference vs. placebo (95% CI)	–3.0 (–5.9 to –0.2)	–2.0 (–4.9 to 0.9)	–3.8 (–6.7 to –1.0)	—
Celiac Disease Questionnaire, overall§				
Baseline				
No. of patients	40	40	35	34
Score	172.5±13.0	164.5±17.5	170.3±12.9	168.4±16.9
After gluten challenge at wk 6				
No. of patients	39	39	36	32
Score	174.5±12.6	166.3±16.6	174.2±13.6	166.4±18.6
Change from baseline (95% CI)†	3.2 (–0.6 to 7.0)	0.9 (–3.0 to 4.7)	3.7 (–0.2 to 7.7)	–2.1 (–6.2 to 2.1)
Estimated difference vs. placebo (95% CI)	5.3 (–0.4 to 10.9)	2.9 (–2.7 to 8.6)	5.8 (0.1 to 11.5)	—
Celiac Disease Questionnaire, gastrointestinal subscore§				
Baseline				
No. of patients	41	40	38	36
Score	42.6±5.6	41.7±4.5	42.0±4.2	41.9±5.3
After gluten challenge at wk 6				
No. of patients	40	41	39	34
Score	42.1±6.5	40.5±5.8	42.3±4.6	38.3±7.0
Change from baseline (95% CI)†	–0.5 (–2.1 to 1.2)	–1.2 (–2.9 to 0.4)	0.1 (–1.6 to 1.8)	–3.6 (–5.4 to –1.8)

Table 3. (Continued.)

Variable	ZED1227, 10 mg	ZED1227, 50 mg	ZED1227, 100 mg	Placebo
Estimated difference vs. placebo (95% CI)	3.1 (0.7 to 5.5)	2.4 (–0.1 to 4.8)	3.7 (1.2 to 6.2)	—

* Plus–minus values are means ±SD. The analyses included all 159 patients who underwent randomization and received at least one dose of ZED1227 or placebo.
† The change from baseline is presented as a least-squares means estimate.
‡ The Celiac Symptom Index is a 16-item questionnaire, with each item rated on a scale of 1 (no symptoms) to 5 (symptoms all the time). Overall scores range from 16 to 80, with higher scores indicating worse celiac disease–related symptoms.
§ The Celiac Disease Questionnaire is a 28-item questionnaire, with each item rated on a scale of 1 (reduced health-related quality of life) to 7 (high health-related quality of life). Overall scores range from 28 to 196, and gastrointestinal subscores from 7 to 49, with higher scores indicating better quality of life.

Table 4. Common Adverse Events That Occurred in Three or More Patients in Any Group.*

Event	ZED1227, 10 mg (N=41)		ZED1227, 50 mg (N=41)		ZED1227, 100 mg (N=40)		Placebo (N=38)	
	Any Event	Related Event	Any Event	Related Event	Any Event	Related Event	Any Event	Related Event
Any adverse event	33 (80)	14 (34)	30 (73)	19 (46)	32 (80)	20 (50)	30 (79)	21 (55)
Headache	9 (22)	6 (15)	13 (32)	7 (17)	10 (25)	4 (10)	13 (34)	6 (16)
Nausea	6 (15)	6 (15)	7 (17)	5 (12)	4 (10)	4 (10)	7 (18)	5 (13)
Diarrhea	4 (10)	2 (5)	5 (12)	3 (7)	6 (15)	5 (12)	4 (11)	2 (5)
Vomiting	4 (10)	2 (5)	3 (7)	3 (7)	1 (2)	1 (2)	8 (21)	7 (18)
Abdominal pain	3 (7)	0	5 (12)	3 (7)	5 (12)	3 (8)	3 (8)	3 (8)
Nasopharyngitis	2 (5)	0	4 (10)	0	4 (10)	0	4 (11)	2 (5)
Abdominal distention	2 (5)	0	4 (10)	3 (7)	4 (10)	4 (10)	3 (8)	2 (5)
Fatigue	2 (5)	1 (2)	6 (15)	3 (7)	2 (5)	2 (5)	3 (8)	3 (8)
Flatulence	3 (7)	1 (2)	4 (10)	4 (10)	1 (2)	1 (2)	3 (8)	3 (8)
Upper abdominal pain	2 (5)	1 (2)	2 (5)	2 (5)	3 (8)	1 (2)	3 (8)	3 (8)
Constipation	2 (5)	2 (5)	3 (7)	3 (7)	1 (2)	1 (2)	1 (3)	1 (3)
Transferrin saturation decrease	3 (7)	0	1 (2)	1 (2)	2 (5)	1 (2)	1 (3)	0
Lipase level increase	0	0	3 (7)	2 (5)	0	0	0	0
Rash	0	0	0	0	3 (8)	3 (8)	0	0

* All adverse events and those that were considered to be related to ZED1227 or placebo are shown. The safety analysis included all 159 patients who underwent randomization and received at least one dose of ZED1227 or placebo. One patient was added to the 100-mg group for the safety analysis because this patient received the trial drug; however, the patient was lost to follow-up, so the administration of the medication was uncertain, and the safety data are not completely available.

dated quantitative histopathological testing as the primary end point in our proof-of-concept trial.[20,21,24,27,29,35-38]

Celiac disease is highly prevalent in White, Middle Eastern, and Indian populations and is prevalent among Native Americans and Northeast Asians. The prevalence is low among Black Africans and Southeast Asians, but the prevalence in these populations can be high if European ancestry is also involved. Therefore, the patients in our trial represented the ancestral cross-section of the European countries participating in the trial; there was no intention to include or exclude certain racial or ethnic groups from the trial.

Strengths of this trial were the high levels of patient adherence to the regimen and to the gluten challenge, which maximized the data that

could be evaluated. Limitations include a substantial amount of missing data and the loss of several patients to follow-up, the short duration of the trial, and the controlled gluten ingestion. Future studies of ZED1227 in more patients are needed to provide additional evidence of the safety and efficacy of the drug, potentially in real-life conditions with minor gluten ingestion.

In this phase 2 trial, we found that the oral transglutaminase 2 inhibitor ZED1227 effectively attenuated intestinal mucosal injury in patients with celiac disease challenged with a moderate dose of daily gluten.

Supported by Dr. Falk Pharma.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank the patients and their families and the trial teams at the participating trial centers; Martin Krauss (statistician), Gerd Bouma (gastroenterologist), and Gino Corazza (gastroenterologist) for serving as members of the independent data monitoring committee; Anneli Vuorinen and Johanna Haikonen (both from Crown CRO) for assistance in conducting the clinical trial; Anni Rava, Kaisa Laapas, and Inger-Helen Maadik (all from StatFinn, an IQVIA company) for data management and statistical expertise; Tiina Kärjä-Lahdensuu (from CO Consult) for local operational and regulatory assistance; Sibylle Neufang (University Medical Center, Mainz, Germany) for local operational assistance; the staff of the Finnish Celiac Society (Keliakialiitto), the German Celiac Society (Deutsche Zöliakiegesellschaft), and the Swiss Celiac Association (IG Zöliakie der Deutschen Schweiz) for generosity and assistance; and Jun Yan (of Jun Yan Medical Writings) for writing the first draft of the manuscript and for providing native-speaker linguistic and grammatical review of earlier versions of the manuscript.

APPENDIX

The authors' full names and academic degrees are as follows: Prof. Detlef Schuppan, M.D., Ph.D., Markku Mäki, M.D., Ph.D., Knut E.A. Lundin, M.D., Ph.D., Jorma Isola, M.D., Ph.D., Tina Friesing-Sosnik, M.D., Juha Taavela, M.D., Ph.D., Alina Popp, M.D., Ph.D., Jari Koskenpato, M.D., Jost Langhorst, M.D., Øistein Hovde, M.D., Ph.D., Marja-Leena Lähdeaho, M.D., Ph.D., Stefano Fusco, M.D., Michael Schumann, M.D., Helga P. Török, M.D., Juozas Kupcinskas, M.D., Yurdagül Zopf, M.D., Ansgar W. Lohse, M.D., Mika Scheinin, M.D., Ph.D., Karin Kull, M.D., Luc Biedermann, M.D., Valerie Byrnes, M.D., Andreas Stallmach, M.D., Jørgen Jahnsen, M.D., Jonas Zeitz, M.D., Ralf Mohrbacher, M.Sc., and Roland Greinwald, Ph.D.

The authors' affiliations are as follows: the Institute of Translational Immunology and Celiac Center, Research Center for Immune Therapy, University Medical Center, Johannes Gutenberg University, Mainz (D.S., T.F.-S.), the Department of Internal and Integrative Medicine, Sozialstiftung Bamberg, Bamberg (J.L.), the Department of Integrative Medicine, University of Duisburg-Essen, Duisburg-Essen (J.L.), the Division of Gastroenterology, Hepatology, and Infectious Diseases, Department of Internal Medicine I, University Hospital Tübingen, Tübingen (S.F.), the Department of Gastroenterology, Infectious Diseases, and Rheumatology, Campus Benjamin Franklin, Charité–University Medicine Berlin, Berlin (M. Schumann), the Department of Medicine II, University Hospital, Ludwig Maximilians University, Munich (H.P.T.), the Department of Medicine 1, Hector Center for Nutrition, Exercise, and Sports, Universitätsklinikum Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen (Y.Z.), the Department of Medicine I, University Medical Center Hamburg-Eppendorf, Hamburg (A.W.L.), the Department of Internal Medicine IV, University Hospital, Friedrich-Schiller University Jena, Jena (A.S.), and Dr. Falk Pharma, Freiburg (R.M., R.G.) — all in Germany; the Division of Gastroenterology and Celiac Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston (D.S.); the Faculty of Medicine and Health Technology, Tampere University and Tampere University Hospital (M.M., A.P., M.-L.L.), the Faculty of Medicine and Health Technology, Tampere University (J.I., J.T.), Jilab (J.I.), and the Department of Pediatrics, Tampere University Hospital (M.-L.L.), Tampere, the Department of Internal Medicine, Central Finland Central Hospital, Jyväskylä (J.T.), Lääkärikeskus Aava Helsinki Kamppi, Helsinki (J. Koskenpato), and Clinical Research Services Turku, Turku (M. Scheinin) — all in Finland; Oslo University Hospital, Rikshospitalet, and Stiftelsen K.G. Jebsen Celiac Disease Research Center, University of Oslo, Oslo (K.E.A.L.), the Medical Department, Innlandet Hospital Trust, Gjøvik (O.H.), and Akershus University Hospital, Lørenskog (J.J.) — all in Norway; the University of Medicine and Pharmacy "Carol Davila" and the National Institute for Mother and Child Health "Alessandrescu-Rusescu," Bucharest, Romania (A.P.); the Gastroenterology Department and Institute for Digestive Research, Lithuanian University of Health Sciences, Kaunas, Lithuania (J. Kupcinskas); the Department of Gastroenterology, Internal Medicine Clinic, Tartu University Hospital, Tartu, Estonia (K.K.); the Department of Gastroenterology and Hepatology, University Hospital Zurich (L.B., J.Z.), and the Swiss Celiac Center, Center of Gastroenterology, Clinic Hirslanden (J.Z.) — both in Zurich, Switzerland; and University College Hospital Galway, Galway, Ireland (V.B.).

REFERENCES

1. King JA, Jeong J, Underwood FE, et al. Incidence of celiac disease is increasing over time: a systematic review and meta-analysis. Am J Gastroenterol 2020;115:507-25.
2. Singh P, Arora A, Strand TA, et al. Global prevalence of celiac disease: systematic review and meta-analysis. Clin Gastroenterol Hepatol 2018;16(6):823.e2-836.e2.
3. Lionetti E, Gatti S, Pulvirenti A, Catassi C. Celiac disease from a global perspective. Best Pract Res Clin Gastroenterol 2015;29:365-79.
4. Lohi S, Mustalahti K, Kaukinen K, et al. Increasing prevalence of coeliac disease over time. Aliment Pharmacol Ther 2007;26:1217-25.
5. Kahaly GJ, Frommer L, Schuppan D. Celiac disease and endocrine autoimmunity — the genetic link. Autoimmun Rev 2018;17:1169-75.
6. Lebwohl B, Sanders DS, Green PHR. Coeliac disease. Lancet 2018;391:70-81.
7. Leffler DA, Green PHR, Fasano A. Extraintestinal manifestations of coeliac disease. Nat Rev Gastroenterol Hepatol 2015;12:561-71.
8. Lundin KE, Sollid LM. Advances in coeliac disease. Curr Opin Gastroenterol 2014;30:154-62.
9. Ludvigsson JF, Leffler DA, Bai JC, et al. The Oslo definitions for coeliac disease and related terms. Gut 2013;62:43-52.
10. Schuppan D, Junker Y, Barisani D. Celiac disease: from pathogenesis to novel therapies. Gastroenterology 2009;137:1912-33.
11. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. Eur J Gastroenterol Hepatol 1999;11:1185-94.

12. Syage JA, Kelly CP, Dickason MA, et al. Determination of gluten consumption in celiac disease patients on a gluten-free diet. Am J Clin Nutr 2018;107:201-7.
13. Hære P, Høie O, Schulz T, Schönhardt I, Raki M, Lundin KE. Long-term mucosal recovery and healing in celiac disease is the rule — not the exception. Scand J Gastroenterol 2016;51:1439-46.
14. Roos S, Liedberg GM, Hellström I, Wilhelmsson S. Persistent symptoms in people with celiac disease despite gluten-free diet: a concern? Gastroenterol Nurs 2019;42:496-503.
15. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797-801.
16. Molberg O, Mcadam SN, Körner R, et al. Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat Med 1998;4:713-7.
17. van de Wal Y, Kooy Y, van Veelen P, et al. Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. J Immunol 1998;161:1585-8.
18. Abadie V, Kim SM, Lejeune T, et al. IL-15, gluten and HLA-DQ8 drive tissue destruction in coeliac disease. Nature 2020;578:600-4.
19. Kuitunen P, Kosnai I, Savilahti E. Morphometric study of the jejunal mucosa in various childhood enteropathies with special reference to intraepithelial lymphocytes. J Pediatr Gastroenterol Nutr 1982;1:525-31.
20. Lähdeaho M-L, Scheinin M, Vuotikka P, et al. Safety and efficacy of AMG 714 in adults with coeliac disease exposed to gluten challenge: a phase 2a, randomised, double-blind, placebo-controlled study. Lancet Gastroenterol Hepatol 2019;4:948-59.
21. Lähdeaho M-L, Kaukinen K, Laurila K, et al. Glutenase ALV003 attenuates gluten-induced mucosal injury in patients with celiac disease. Gastroenterology 2014;146:1649-58.
22. Leffler D, Schuppan D, Pallav K, et al. Kinetics of the histological, serological and symptomatic responses to gluten challenge in adults with coeliac disease. Gut 2013;62:996-1004.
23. Lähdeaho M-L, Mäki M, Laurila K, Huhtala H, Kaukinen K. Small-bowel mucosal changes and antibody responses after low- and moderate-dose gluten challenge in celiac disease. BMC Gastroenterol 2011;11:129.
24. Taavela J, Koskinen O, Huhtala H, et al. Validation of morphometric analyses of small-intestinal biopsy readouts in celiac disease. PLoS One 2013;8(10):e76163.
25. Leffler DA, Dennis M, Edwards George J, et al. A validated disease-specific symptom index for adults with celiac disease. Clin Gastroenterol Hepatol 2009;7(12):1328-34, 1334.e1-1334.e3.
26. Häuser W, Gold J, Stallmach A, Caspary WF, Stein J. Development and validation of the Celiac Disease Questionnaire (CDQ), a disease-specific health-related quality of life measure for adult patients with celiac disease. J Clin Gastroenterol 2007;41:157-66.
27. Taavela J, Viiri K, Popp A, et al. Histological, immunohistochemical and mRNA gene expression responses in coeliac disease patients challenged with gluten using PAXgene fixed paraffin-embedded duodenal biopsies. BMC Gastroenterol 2019;19:189.
28. Hindryckx P, Levesque BG, Holvoet T, et al. Disease activity indices in coeliac disease: systematic review and recommendations for clinical trials. Gut 2018;67:61-9.
29. Ludvigsson JF, Ciacci C, Green PH, et al. Outcome measures in coeliac disease trials: the Tampere recommendations. Gut 2018;67:1410-24.
30. Leffler D, Kupfer SS, Lebwohl B, et al. Development of celiac disease therapeutics: report of the Third Gastroenterology Regulatory Endpoints and Advancement of Therapeutics Workshop. Gastroenterology 2016;151:407-11.
31. Mäki M, Lähdeaho ML, Hällström O, Viander M, Visakorpi JK. Postpubertal gluten challenge in coeliac disease. Arch Dis Child 1989;64:1604-7.
32. Lebwohl B, Granath F, Ekbom A, et al. Mucosal healing and risk for lymphoproliferative malignancy in celiac disease: a population-based cohort study. Ann Intern Med 2013;159:169-75.
33. Rubio-Tapia A, Rahim MW, See JA, Lahr BD, Wu T-T, Murray JA. Mucosal recovery and mortality in adults with celiac disease after treatment with a gluten-free diet. Am J Gastroenterol 2010;105:1412-20.
34. Kaukinen K, Peräaho M, Lindfors K, et al. Persistent small bowel mucosal villous atrophy without symptoms in coeliac disease. Aliment Pharmacol Ther 2007;25:1237-45.
35. Daveson AJM, Popp A, Taavela J, et al. Baseline quantitative histology in therapeutics trials reveals villus atrophy in most patients with coeliac disease who appear well controlled on gluten-free diet. GastroHep 2020;2:22-30.
36. Adelman DC, Murray J, Wu T-T, Mäki M, Green PH, Kelly CP. Measuring change in small intestinal histology in patients with celiac disease. Am J Gastroenterol 2018;113:339-47.
37. Goel G, King T, Daveson AJ, et al. Epitope-specific immunotherapy targeting CD4-positive T cells in coeliac disease: two randomised, double-blind, placebo-controlled phase 1 studies. Lancet Gastroenterol Hepatol 2017;2:479-93.
38. Hamilton G, Mäki M, Lahdeaho M-l, Bhasin M, Bekker P, Schall TJ. A randomized, double-blind, placebo-controlled, phase II study testing CCX282-B in the treatment of celiac disease. Gastroenterology 2008;134:Suppl. 1:A-493. abstract.