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A Randomized Trial of a Transglutaminase 2 Inhibitor for Celiac Disease

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Abstract

BACKGROUND: In celiac disease (CeD), small intestinal transglutaminase 2 (TG2) deamidates glutamine residues in gluten peptides, which enhances stimulation of T cells and leads to mucosal injury. TG2 inhibition is a potential treatment for CeD.

METHODS: This proof-of-concept study assessed the efficacy and safety of a 6-week treatment with 3 doses of ZED1227, a selective oral TG2 inhibitor, versus placebo in 159 adult caucasian patients with well controlled CeD who were challenged with gluten. Primary endpoint was attenuation of gluten-induced mucosal damage, measured by villus height-to-crypt depth ratio. Secondary endpoints included intraepithelial lymphocyte density, the Celiac Symptom Index and Celiac Disease Questionnaire (assessing health-related quality of life).

RESULTS: Of 41, 41, 41, and 40 patients assigned to the 10 mg, 50 mg, 100 mg ZED1227 and placebo groups, respectively, 35, 39, 38, and 30 had adequate duodenal biopsies for primary outcome assessment. All 3 doses attenuated gluten-induced duodenal mucosal injury. The estimated differences (95% confidence interval) in the change of mean villus height-to-crypt ratio from baseline to week 6 for 10 mg, 50 mg, and 100 mg ZED1227 versus placebo were 0.44 (0.150, 0.727; P=0.0010), 0.49 (0.204, 0.767; P=0.0002), and 0.48 (0.196, 0.766; P=0.0002), respectively. The estimated differences in the change of intraepithelial lymphocyte density were -2.7 (-7.58, 2.23), -4.2 (-8.94, 0.63), and -9.6 (-14.38, -4.75), respectively. The 100 mg dose may have improved symptom and quality of life scores. The most common adverse events, similar across all groups, were headache, nausea, diarrhea, vomiting, and abdominal pain. Three patients (7.5%) in the 100 mg group developed rash.

CONCLUSION: In this preliminary trial, ZED1227 attenuated gluten-induced duodenal mucosal damage in patients with celiac disease. (EudraCT No.: 2017-002241-30)

Background

Celiac disease (CeD) is characterized by small intestinal inflammation, is frequently associated with autoimmunity, and affects 0.2% to 2% of most countries' populations.[1-3] Identified cases have increased due to improved serological diagnosis, as has true CeD prevalence in the past decades.[1,4] CeD is driven by the ingestion of gluten in wheat and related grains in genetically predisposed individuals who are carriers of human leukocyte antigen types HLA-DQ2 and HLA-DQ8, which are necessary but not sufficient for the manifestation of CeD. The classical symptoms of CeD are diarrhea, weight loss, and malnutrition, but CeD 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 CeD is diagnosed by elevated serum autoantibodies to transglutaminase 2 (TG2) and confirmed by histological villus atrophy and crypt hyperplasia in the proximal small intestine, accompanied by duodenal mucosal intraepithelial lymphocyte (IEL) infiltration.[11] The only available treatment for CeD is life-long adherence to a strict gluten-free diet (GFD), a diet which is difficult to maintain[12], and only 50% of patients show mucosal recovery and often do not achieve negative serum autoantibodies one year or later after diagnosis.[13] Moreover, many CeD patients report persistent symptoms despite their adherence to the GFD.[14] Thus, there is an unmet medical need for an effective treatment adjunct to an even strict GFD. Currently, no drug therapy reliably prevented the effects of dietary gluten nor has been approved by regulators to treat CeD.

TG2, the celiac autoantigen[8,15-17] is expressed in the intestinal mucosa where it modifies immunogenic gluten peptides through deamidation of certain charge-neutral glutamine residues,

yielding negatively charged glutamic acid residues.[16,17] This modification promotes gluten-peptide presentation by HLA-DQ2 or -DQ8 molecules on mucosal antigen presenting cells[8,10] and enables the activation and expansion of gluten peptide-specific CD4+ T helper 1 cells and the secretion of pro-inflammatory cytokines. This leads to villus atrophy and crypt hyperplasia and to B cell differentiation and the production of Immunoglobulin A (IgA)-TG2.[8,10,15-17]

We designed ZED1227, which inhibits TG2 with a 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, 2015-005283-42). Our phase 1 clinical studies in 106 healthy subjects exposed to doses of up to 500 mg of ZED1227 for up to 8 days have not shown drug-related adverse effects or signs of toxicity, with low systemic drug levels after oral ingestion. Here, we report the results of a phase 2, double-blind, placebo-controlled efficacy, safety, and dose-finding study of ZED1227 capsules in adult patients with CeD in histological and serological (IgA-TG2) remission on gluten free diet who were challenged with daily gluten for 6 weeks.

Methods

This study was conducted at 20 sites in 7 countries (Estonia, Finland, Germany, Ireland, Lithuania, Norway, Switzerland) from May 16, 2018 to February 27, 2020. The study was approved by independent ethics committees at each center. Written informed consent was obtained from each patient before screening.

Trial Patients

Adults 18 to 65 years of age with biopsy-confirmed diagnosis of CeD at least 12 months before screening and positivity for HLA-DQ2 and/or -DQ8 genotypes were considered for study inclusion. Patients had to have successfully adhered to the gluten free diet for at least 12 months, present with a negative IgA-TG2 serology and a mean villus height-to-crypt depth ratio (VH:CrD) of 1.5 or higher, and agree to tolerate a challenge of ingesting 3 grams of gluten daily for 6 weeks. Full inclusion and exclusion criteria are provided in the Supplementary Appendix.

Trial Design and Treatment

In this randomized, double-blind, placebo-controlled, dose-ranging study (see study design and execution schematic in Supplementary Fig. S1) eligible patients were randomized, in a 1:1:1:1 ratio, to one of 4 parallel groups and received treatment with 10 mg, 50 mg, or 100 mg ZED1227, or placebo, all with matched appearance, concurrent with gluten challenge. Each morning after at least 6 hours of fasting, patients took the study drug orally and, 30 minutes later, one sponsor-provided biscuit containing 3 grams of gluten, before breakfast. Throughout the 6-week study, patients were required to continue their strict gluten free diet.

Duodenal mucosal damage as objective marker of gluten-induced CeD activity can be measured quantitatively with standardized histopathological morphometry.[19] Gluten challenge, in which a moderate amount of gluten is ingested daily for a limited duration, produces a significant but reversible duodenal mucosal deterioration and allows efficacy assessment of active treatment.[20-23]

Endpoints

The primary endpoint was whether ZED1227 could attenuate gluten-induced deterioration of intestinal mucosal morphology, as measured by the VH:CrD in duodenal biopsy samples, from baseline (at screening) to the end of the 6-week gluten challenge and treatment

period.[24] The secondary endpoints included changes from baseline to week 6 in the density of CD3-positive IELs, the modified Marsh-Oberhuber classification,[11] patient-reported outcomes (PROs) as measured by the Celiac Symptom Index (CSI)[25] and Celiac Disease Questionnaire (CDQ),[26] blood markers of malabsorption (e.g., ferritin, transferrin saturation, albumin), plasma concentrations of ZED1227, and serological markers of CeD. The CSI is a 16-item questionnaire, with each item rated on a scale of 1 to 5, ranging from no symptoms to symptoms all the time. The CDQ is a 28-item questionnaire, with each item rated from 1 to 7, ranging from highly reduced to excellent health-related quality of life. Safety was evaluated by the monitoring of adverse events (AEs), vital signs, body weight, clinical laboratory tests, and tolerability assessed by the investigator and the patient. For assessment of changes of VH:CrD patients were excluded if they did not have adequate follow-up duodenal biopsy samples allowing measurement of at least three separate villus-crypt units.[24] Sensitivity analysis was performed for randomized patients who received at least one dose of study drug. All other efficacy analyses were performed in randomized patients who received at least one dose of study drug without imputing missing values at follow-up; modelling was performed on complete cases only.

Trial Procedures and Assessments

Patient enrolment required a screening period of no more than 8 weeks, including upper gastrointestinal endoscopy with duodenal biopsies within 4 weeks before the first dose administration to provide baseline histological data. At the week 0 visit, the study drug (ZED1227 or placebo) and gluten biscuits were dispensed to patients according to their treatment allocation. Patients returned to the study sites at weeks 2, 4, and 6 for assessments and 10 for a follow-up visit. A second endoscopy with biopsies was performed at the week 6 or withdrawal visit, both endoscopies being conducted by experienced gastroenterologists. 4 forceps biopsies

(one biopsy per pass) were taken 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 both processing and reading. Validated, standardized morphometry procedures separately evaluated mucosal morphology and inflammation.[24,27] The categorical Marsh-Oberhuber classification[11] was used for standard classification of the mucosal lesions (detailed in the Supplementary Appendix).

Patients kept a diary (paper or electronic) to record daily study drug, gluten biscuit, and food intake, concomitant medications, and stool frequency and characteristics. CSI[25] and CDQ[26] scores were determined at all visits. At end of treatment (week 6), investigators and patients independently rated the study treatment as "very good, good, satisfactory, or poor" for both efficacy and tolerability.

AEs were recorded and evaluated by the investigators. Blood was drawn to determine hematology, coagulation, and serum markers.

Statistical Analysis

We estimated that a sample size of 136 patients, or 34 per treatment group, would provide 80% power for the primary analysis, assuming an α error of 0.05, an effect size of 0.6, and standard deviation (SD) of 0.8. With an estimated dropout and non-evaluable rate of 15%, a total of 160 patients (40 per group) were planned for study enrolment.

The primary endpoint, mean change in VH:CrD from baseline to week 6, was analyzed using a generalized linear model with the identity link, in which treatment group and baseline VH:CrD were fixed effects, as assumptions for the parametric model were met. Each ZED1227 dose group was compared with placebo. The same statistical method was used for the analyses of change in IEL density, CSI scores and CDQ scores at week 6 from baseline, which were key

secondary endpoints. Statistics for other endpoints (change of Marsh-Oberhuber classification, IgA-TG2 and anti-endomysial (IgA-EmA) antibodies, and blood malabsorption markers) are described in Supplementary Appendix. Analysis to assess sensitivity to missing data is described in Supplementary Appendix.

Plasma concentrations of ZED1227 and metabolites were measured and analyzed for pharmacokinetic profiles. The results will be reported separately.

All statistical comparisons between each ZED1227 dose and placebo were two-sided with a family-wise alpha error level of 0.05. For the primary endpoint, 95% CI and P-values were adjusted for multiple comparisons using Bonferroni correction to account for the 3 comparisons with placebo. The adjusted P-value required to declare statistical significance for the primary outcome was 0.0167, and individual confidence intervals were constructed using 98.3% levels. For secondary outcomes, 95% CIs are reported without p values; the 95% CI 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 (Supplementary Appendix).

All authors vouch for the accuracy and completeness of the data. The trial was conducted and reported according to the pre-specifications in the protocol, which is available at nejm.org.

Results

Patient Characteristics and Compliance

Of the 163 patients randomized, 3 were not dispensed study medication because of the development of other clinical conditions, and 1 was dispensed medication but was lost to follow up (Supplementary Fig. S2). Analyses of efficacy excluded these 4 patients and thus included 41,

41, 39, and 38 patients in the 10 mg, 50 mg, 100 mg ZED1227, and placebo groups, respectively. The demographic characteristics were comparable across the groups, except for a higher proportion of female patients on 10 mg ZED1227 (Table 1). According to investigators' assessment and patient diaries, compliance rates were high and similar in all 4 groups, ranging from 96% to 100% for both study drug and gluten intake.

Efficacy Results

Histology-related efficacy endpoints could be evaluated in 142 patients who had sufficient biopsy samples at both baseline and week 6, including 35, 39, 38, and 30 patients in the 10 mg, 50 mg, 100 mg ZED1227 and placebo groups, respectively. 4 of these patients stopped treatment before week 6, but qualified for primary outcome analysis with treatment duration from 23 to 32 days. As expected, the gluten challenge decreased the VH:CrD from baseline to week 6 in the placebo group (estimate -0.61; 95% CI -0.781, -0.435). Daily doses of 50 mg and 100 mg ZED1227 prevented this deterioration (estimates -0.12 and -0.13; 95% CIs -0.274, 0.028 and -0.281, 0.026, respectively), with slightly less efficacy in the 10 mg dose group (estimate -0.17; 95% CI -0.329, -0.010). Compared to placebo, all 3 doses of ZED1227 significantly prevented the decrease of the VH:CrD ($P \leq 0.0010$ for all comparisons) (Table 2, Fig. 1, Supplementary Fig. S3a). Sensitivity analysis did not change the result (Supplementary Table S1).

Gluten ingestion caused an increase from baseline in IEL density, a key parameter of mucosal inflammation, in the placebo group and the 10 mg and 50 mg, but not in the 100 mg ZED1227 group, and the increase was attenuated dose-dependently by ZED1227 (Table 3, Supplementary Fig. S4, S3b). Using the modified Marsh-Oberhuber classification, a histological score where 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 3 doses of ZED1227 over placebo (Supplementary Tables S2, S3).

The CSI score, ranging from 16 to 80, with higher scores indicating worse CeD-related symptoms, increased from baseline to week 6 in all groups and returned to baseline at the follow-up visit. Comparison with placebo favored all doses of ZED1227 (Table 3, Supplementary Fig. S5A). The total CDQ score, ranging from 28 to 196, with higher scores indicating better quality of life, increased from baseline to week 6 in all ZED1227 dose groups, but decreased with placebo (Table 3, Supplementary Fig. S5B). Similarly, the changes in the CDQ gastrointestinal subscore (range from 7 to 49) from baseline to week 6 and comparison with placebo favored ZED1227 (Table 3, Supplementary Fig. S5C). At the week 6 visit, the patients' own assessment of "poor efficacy" was 9.8%, 7.3%, 7.7%, and 26.3% of patients in the 10 mg, 50 mg, 100 mg ZED1227 and placebo groups, respectively (Supplementary Table S4).

At screening, all patients had normal baseline IgA-TG2 levels. At week 6, 1 (2.4%), 1 (2.4%), and 0 patients who received 10 mg, 50 mg, 100 mg ZED1227, respectively, converted to positive, compared with 6 (15.8%) placebo patients. The titer of IgA-TG2 increased by a mean of 2.4, 1.7, and 0.3 kU/L in the placebo, 10 mg, and 50 mg groups, respectively, and decreased by 0.1 kU/L in the 100 mg group. Comparable results were obtained for IgA-EmA (Supplementary Table S5, S6).

Safety

AEs occurred in 125 (78.1%) of the 160 patients who received at least one dose of ZED1227 or placebo (Table 4). Most AEs were apparently related to the gluten challenge. 74 (46.3%) patients had AEs that the investigators rated as potentially related to the study drug, including 14 (34.1%), 19 (46.3%), 20 (50.0%), and 21 (55.3%) patients in the ZED1227 10 mg,

50 mg, 100 mg, and placebo groups, respectively. The most common AEs across all groups were headache, nausea, diarrhea, vomiting, and abdominal pain. With the exception of rash, which occurred in 3 patients (7.5%) only in the 100 mg ZED1227 group, no AEs appeared to be more common in the ZED1227 groups than in the placebo group. Serious AEs that were considered related to the study drug occurred in 2 patients: one patient taking 50 mg ZED1227 had migraine with aura, and the other taking placebo had ventricular extrasystoles. Both patients discontinued study treatment and recovered.

A broad range of parameters were measured in blood, including cell counts, liver and kidney functions, surrogates of resorption (albumin, transferrin saturation, zinc, vitamin B12, folic acid), and factor XIII, another common transglutaminase. Results were comparable across the ZED1227 and placebo groups. Notably, during gluten challenge alanine aminotransferase and alkaline phosphatase values increased from baseline to week 6 in the placebo group, which normalized at week 10. These changes were not observed in any of the ZED1227 dose groups (Supplementary Table S7).

Discussion

In this preliminary, randomized, double-blind, placebo-controlled, 6-week study, the effectiveness of TG2 inhibition with the oral TG2 inhibitor ZED1227 was demonstrated in patients with CeD who were challenged with a moderate amount (3 grams) of daily gluten intake. ZED1227 has been developed to specifically block TG2-mediated potentiation of gluten-peptide immunogenicity in the small intestinal mucosa, a key driver of CeD pathogenesis.[8,9] Compared with placebo, all doses of ZED1227 attenuated the gluten-induced small intestinal damage.

This study showed that treatment with the highly specific TG2 inhibitor ZED1227 attenuated the gluten-induced damage in the duodenal mucosa. Importantly, the VH:CrD is widely considered as the most objective and valid primary endpoint in clinical studies for CeD therapies,[24,28-30] and the endpoint was achieved at all 3 doses of ZED1227. The benefits across multiple endpoints 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 CeD. Overall, adverse events were similar in the ZED1227 and placebo groups.

Since the discovery of TG2 as the autoantigen in CeD,[17] extensive research has confirmed TG2 as a crucial mechanistic driver of gluten-induced inflammation and clinical manifestations in CeD.[8,10,15,16] This study supports the role of TG2 in CeD pathogenesis, as its inhibition prevents the deamidation of gluten peptides in the small intestinal mucosa and thus abolishes the immunogenic process. ZED1227 predominantly targets the intestinal mucosa and thereby mediates protection; thus, it is unaffected by the complexity of the food matrix and less dependent on timing of the ingestion of gluten-containing food.

The Food and Drug Administration recently reinforced that, in pharmacological CeD trials prevention of histological damage should be a major endpoint in phase 2 clinical studies, and improvement of CeD-related PROs and quality of life be (co-)primary endpoints in phase 3 trials.[30] This is justified, since only 40% of newly diagnosed adult patients have gastrointestinal symptoms, while a gluten challenge induces a manifest duodenal mucosal lesion, often before any symptoms occur.[22,31] Further, mucosal healing is considered the key criterion of successful treatment and a prerequisite for patients' long-term wellbeing and the prevention of severe

complications.[32-34] Therefore, we selected gluten challenge and used validated quantitative histopathology as the primary outcome in our proof-of-concept study.[20,21,24,27,29,35-38]

A strength of this trial were the high levels of patient compliance with study drug and gluten challenge maximizing evaluable data. Limitations include substantial rates of loss to follow up and missing data, the short study duration, and the controlled gluten ingestion. Future studies of ZED1227 in more patients are needed to provide additional evidence of the drug's safety and efficacy, potentially in real-life conditions with minor gluten ingestion.

In conclusion, the oral TG2 inhibitor ZED1227 effectively attenuated intestinal mucosal injury in patients with celiac disease challenged with a moderate dose of daily gluten.

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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;16:823-36 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 PH, 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. Haere P, Hoie O, Schulz T, Schonhardt 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, Hellstrom I, Wilhelmsson S. Persistent Symptoms in People With Celiac Disease Despite Gluten-Free Diet: A Concern? Gastroenterol Nurs 2019;42:496-503.

15. Molberg O, McAdam SN, Korner 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.

16. 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.

17. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797-801.

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 ML, 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 ML, 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 ML, 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: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;7:1328-34, 34 e1-3.

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, Hallstrom 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 TT, 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, Peraaho 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 TT, 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. T1143 A Randomized, Double-Blind, Placebo-Controlled, Phase II Study Testing CCX282-B in the Treatment of Celiac Disease. Gastroenterology 2008;134.

Figure legends

Figure 1. Paired data plots of villus height-to-crypt depth ratio for global mean

(prespecified analysis population for the primary outcome)

This population included 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 baseline (conducted during screening) and final or withdrawal visits (N=142). Horizontal lines indicate mean values. VH:CrD: villus height-to-crypt depth ratio.

Table 1. Patient demographics

Characteristic	10 mg ZED1227 (N=41)	50 mg ZED1227 (N=41)	100 mg ZED1227 (N=39)	Placebo (N=38)
Age, years, mean (SD)	40.2 (12.4)	42.8 (12.1)	41.0 (14.8)	42.5 (14.4)
Female sex, no. (%)	37 (90.2)	29 (70.7)	24 (61.5)	28 (73.7)
Race, white, no. (%)	41 (100)	41 (100)	39 (100)	38 (100)
Mean weight, kg, mean (SD)	70.9 (12.9)	71.8 (13.1)	73.2 (13.7)	68.4 (14.7)

This table includes 159 of the 163 randomized patients; 3 patients who were not dispensed study medication and 1 patient who was lost to follow up were excluded from efficacy analysis.

Table 2. Effect of ZED1227 on villus height-to-crypt depth ratio

	10 mg ZED1227 (N=35)	**50 mg ZED1227 (N=39)**	**100 mg ZED1227 (N=38)**	**Placebo (N=30)**
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Baseline	2.013 (0.298)	2.041 (0.316)	2.089 (0.346)	1.975 (0.334)
Post gluten challenge (week 6)	1.851 (0.530)	1.914 (0.436)	1.939 (0.483)	1.390 (0.613)
Change from baseline (95% CI)*	-0.17 (-0.329, -0.010)	-0.12 (-0.274, 0.028)	-0.13 (-0.281, 0.026)	-0.61 (-0.781, -0.435)
Estimated difference relative to placebo (95% CI)**	0.44 (0.105, 0.727)	0.49 (0.204, 0.767)	0.48 (0.196, 0.766)	--
P-value	0.0010	0.0002	0.0002	--

As stipulated in the study protocol the primary analysis of the primary outcome included the 142 randomized patients who 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 baseline (conducted during screening) and final or withdrawal visits. N=17 patients (6, 2, 1, and 7 in the 10 mg, 50 mg, 100 mg and the placebo groups respectively, were excluded because they did not undergo final endoscopy; 1 patient in the placebo group was excluded because one sample obtained was not adequate for analysis). CI: confidence interval

* Least square means estimate

** The Bonferroni correction has been used for 95% CI and P-value adjustment, when estimating treatment difference. The adjusted P-value required to declare statistical significance for the primary outcome was 0.0167, and individual confidence intervals were constructed using 98.3% levels.

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Table 3. Effect of ZED1227 on intraepithelial lymphocytes density, CSI, CDQ

	10 mg ZED1227	**50 mg ZED1227**	**100 mg ZED1227**	**Placebo**
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Intraepithelial lymphocytes density (cells/100 epithelial cells)				
Baseline, n	41	41	39	38
Baseline	26.5 (6.8)	29.3 (9.0)	26.4 (8.4)	27.9 (10.2)
Week 6, n	35	39	38	31
Post gluten challenge (week 6)	34.6 (12.0)	35.7 (12.0)	27.9 (7.8)	38.6 (15.7)
Change from baseline (95% CI)*	8.3 (4.97, 11.70)	6.9 (3.66, 10.06)	1.5 (-1.78, 4.68)	11.0 (7.45, 14.58)
Estimated difference relative to placebo (95% CI)	-2.7 (-7.58, 2.23)	-4.2 (-8.94, 0.63)	-9.6 (-14.38, -4.75)	--
Celiac Symptom Index (CSI)				
Baseline, n	38	39	38	33
Baseline	24.4 (5.6)	27.0 (7.8)	24.2 (5.1)	26.0 (5.8)
Week 6, n	38	38	37	32
Post gluten challenge (week 6)	25.9 (6.1)	29.0 (8.0)	25.2 (5.8)	29.8 (9.4)
Change from baseline (95% CI)*	0.9 (-1.03, 2.85)	2.0 (0.00, 3.91)	0.1 (-1.82, 2.07)	4.0 (1.83, 6.07)
Estimated difference relative to placebo (95% CI)	-3.0 (-5.92, -0.16)	-2.0 (-4.87, 0.88)	-3.8 (-6.71, -0.95)	--
Celiac Disease Questionnaire (CDQ)				
Baseline, n	40	40	35	34

Baseline	172.48 (13.02)	164.51 (17.51)	170.31 (12.92)	168.35 (16.93)
Week 6, n	39	39	36	32
Post gluten challenge (week 6)	174.53 (12.56)	166.29 (16.64)	174.25 (13.64)	166.38 (18.62)
Change from baseline (95% CI)*	3.2 (-0.57, 6.97)	0.9 (-2.96, 4.72)	3.7 (-0.22, 7.70)	-2.1 (-6.20, 2.09)
Estimated difference relative to placebo (95% CI)	5.3 (-0.35, 10.86)	2.9 (-2.71, 8.58)	5.8 (0.07, 11.53)	--
CDQ, subscore gastrointestinal				
Baseline, n	41	40	38	36
Baseline	42.57 (5.63)	41.70 (4.51)	42.03 (4.25)	41.92 (5.30)
Week 6, n	40	41	39	34
Post gluten challenge (week 6)	42.06 (6.47)	40.54 (5.83)	42.33 (4.62)	38.29 (7.04)
Change from baseline (95% CI)*	-0.5 (-2.12, 1.15)	-1.2 (-2.86, 0.44)	0.1 (-1.56, 1.79)	-3.6 (-5.37, -1.78)
Estimated difference relative to placebo (95% CI)	3.1 (0.66, 5.53)	2.4 (-0.07, 4.81)	3.7 (1.24, 6.15)	--

Analyses included all randomized patients who received at least 1 dose of study medication (N=159). CI: confidence interval.
* Least square means estimate
CSI: 16-item questionnaire, each item was rated on a scale of 1 to 5, with 1 indicating no symptoms and 5 indicating symptoms all the time.
CDQ: 28-item questionnaire, each item was rated on a scale of 1 to 7, with 1 indicating reduced and 7 indicating high health-related quality of life.

Table 4. Common adverse events (AEs) that occurred in 3 or more patients in any treatment group

	Number of Patients (%)							
	10 mg ZED1227 (N=41)		50 mg ZED1227 (N=41)		100 mg ZED1227 (N=40)*		Placebo (N=38)	
	Any	Related	Any	Related	Any	Related	Any	Related
Patients with at least one AE	33 (80.5)	14 (34.1)	30 (73.2)	19 (46.3)	32 (80.0)	20 (50.0)	30 (78.9)	21 (55.3)
Headache	9 (22.0)	6 (14.6)	13 (31.7)	7 (17.1)	10 (25.0)	4 (10.0)	13 (34.2)	6 (15.8)
Nausea	6 (14.6)	6 (14.6)	7 (17.1)	5 (12.2)	4 (10.0)	4 (10.0)	7 (18.4)	5 (13.2)
Diarrhea	4 (9.8)	2 (4.9)	5 (12.2)	3 (7.3)	6 (15.0)	5 (12.5)	4 (10.5)	2 (5.3)
Vomiting	4 (9.8)	2 (4.9)	3 (7.3)	3 (7.3)	1 (2.5)	1 (2.5)	8 (21.1)	7 (18.4)
Abdominal pain	3 (7.3)	0	5 (12.2)	3 (7.3)	5 (12.5)	3 (7.5)	3 (7.9)	3 (7.9)
Nasopharyngitis	2 (4.9)	0	4 (9.8)	0	4 (10.0)	0	4 (10.5)	2 (5.3)
Abdominal distension	2 (4.9)	0	4 (9.8)	3 (7.3)	4 (10.0)	4 (10.0)	3 (7.9)	2 (5.3)
Fatigue	2 (4.9)	1 (2.4)	6 (14.6)	3 (7.3)	2 (5.0)	2 (5.0)	3 (7.9)	3 (7.9)
Flatulence	3 (7.3)	1 (2.4)	4 (9.8)	4 (9.8)	1 (2.5)	1 (2.5)	3 (7.9)	3 (7.9)
Abdominal pain upper	2 (4.9)	1 (2.4)	2 (4.9)	2 (4.9)	3 (7.5)	1 (2.5)	3 (7.9)	3 (7.9)
Constipation	2 (4.9)	2 (4.9)	3 (7.3)	3 (7.3)	1 (2.5)	1 (2.5)	1 (2.6)	1 (2.6)
Transferrin saturation decreased	3 (7.3)	0	1 (2.4)	1 (2.4)	2 (5.0)	1 (2.5)	1 (2.6)	0
Lipase increased	0	0	3 (7.3)	2 (4.9)	0	0	0	0
Rash	0	0	0	0	3 (7.5)	3 (7.5)	0	0

Analyses included all randomized patients who received at least 1 dose of study medication (N=159).

* One patient added: Study medication dispensed but lost to follow-up. Medication administration uncertain. Safety data incompletely available.

Data Sharing Statement

Schuppan D, Mäki M, Lundin K, et al. Transglutaminase Inhibitor Attenuates Pathology and Symptoms in Celiac Disease.

Question	Authors' Response
Will the data collected for your study be made available to others?	Yes
Would you like to offer context for your decision?	—
Which data?	Other (eg, partial data sets) — please describe
Additional information about data	Dr. Falk Pharma GmbH is in the process of developing a formal data sharing plan; requests for future data sharing can be sent to zentrale@drfalkpharma.de
How or where can the data be obtained?	Dr. Falk Pharma GmbH is in the process of developing a formal data sharing plan; requests for future data sharing can be sent to zentrale@drfalkpharma.de
When will data availability begin?	After publication and marketing authorization in US & Europe (or development discontinued)
When will data availability end?	There is no end date for eligibility to submit a data sharing request for these data
Will any supporting documents be available?	—
Which supporting documents?	—
Additional information about supporting documents	—
How or where can supporting documents be obtained?	—
When will supporting documents availability begin?	—
When will supporting documents availability end?	—
To whom will data be available?	—
For what type of analysis or purpose?	—
By what mechanism?	—
Any other restrictions?	—
Additional information	—