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Latiglutenase treatment for celiac disease: symptom and quality of life improvement for seropositive patients on a gluten-free diet

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Summary

Background: Celiac disease (CD) is a widespread autoimmune disease triggered by dietary gluten that can lead to severe gastrointestinal symptoms. As there is no available treatment other than a lifelong gluten-free diet, many patients continue to experience chronic symptoms.

Aim: In this analysis we report on the efficacy of latiglutenase, an orally administered enzyme treatment, for improving multiple gluten-induced symptoms and consequent quality of life (QOL) due to inadvertent gluten consumption.

Methods: This analysis is based on data from the CeliAction study of symptomatic patients (ALV003-1221; NCT01917630). Patients were treated with latiglutenase or placebo for 12 weeks and instructed to respond to a symptom diary daily and to multiple QOL questionnaires at weeks 0, 6 and 12 of the treatment periods as secondary endpoints. The results were stratified by serostatus.

Results: 398 patients completed the 12-week CDSD study. In seropositive (but not seronegative) CD patients a statistically significant and dose-dependent improvement was seen in the severity and frequency of abdominal pain, bloating, tiredness and constipation. In subjects receiving 900 mg latiglutenase, improvements (*P*-values) in the severity of these symptoms for week 12 were 58% (0.038), 44% (0.023), 21% (0.164) and 104% (0.049) respectively, relative to placebo-dosed subjects. The reduction in symptoms trended higher for more symptomatic patients. Similar results were observed for the QOL outcome measures.

Conclusions: Although this study was not powered to definitively establish the benefit of latiglutenase in seropositive CD patients, such patients appear to show symptomatic and QOL benefit from using latiglutenase with meals.

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1 | INTRODUCTION

Celiac disease (CD) is a chronic inflammatory disorder of the small intestine triggered by exposure to gluten proteins and affecting about 1% of most populations.[1-3] The pathological lesion of villous atrophy in the proximal epithelium of the small intestine is due to an immune response to wheat, rye or barley. The treatment of CD has been limited to a lifelong gluten-free diet (GFD) which can control but does not cure the disease. While treatment can ameliorate symptoms and damage, the diet is not easy or readily achievable by many patients.[4,5] Low levels of gluten exposure are common and may cause pain and suffering and ongoing inflammation that can increase the risk of complications including lymphoma, bowel cancer, osteoporosis, anaemia, malnutrition, etc.[6,7] Patients and families often have a substantial burden to bear to achieve the diet. Furthermore, the cost of care for moderately to severely symptomatic patients, comprising nearly 50% of patients, is more than $10K/year.[8]

There are several experimental targets for CD in clinical trials,[9] however, to our knowledge sizable randomised drug trials have only been published for two modes of action—dietary enzyme supplementation therapy[10,11] and tight junction modulation in the small intestine.[12,13] Latiglutenase (IMGX003, formerly ALV003) is a novel enzyme supplementation therapy comprised of two enzymes that was recently shown to mitigate gluten-induced mucosal injury in CD patients in a gluten-challenge study (ALV003-1021).[10] A subsequent "real-world" trial (ALV003-1221), however, did not show evidence of treatment-induced mucosal healing relative to placebo due to what was reported to be due to a trial (Hawthorne) effect, in which the patients changed their behaviour during the treatment period by further reducing their gluten intake from their normal GFD.[14] In this same study, however, it was shown that statistically and clinically significant reduction in multiple gluten-induced symptoms was observed as a function of latiglutenase dose in a subpopulation of patients who remained seropositive despite being on a GFD for at least 1 year.[15]

The pharmacological rationale for latiglutenase therapy in CD is conceptually straightforward. Most immunotoxic gluten peptides are highly resistant to proteolytic activity in the intestine.[16] In turn, proteolytic resistance leads to the accumulation of long, metastable gluten-derived intermediates in the small intestinal lumen, which elicit a T-cell dependent response in CD patients. Based on a variety of in vitro, in vivo animal, and ex vivo human studies, it has been suggested that giving exogenous proteases that target the gluten in food could reduce the immunogenic peptides present after gluten exposure and perhaps have a therapeutic role in managing celiac disease.[17]

In this article, we expand on the preliminary symptom analysis presented earlier[15] by presenting additional data and analysis including daily symptom data showing the nature of symptoms manifesting as acute flares that are significantly attenuated by latiglutenase as well as representation of symptom relief in terms of responder analysis relating the percentage of patients who improve by threshold amounts while on treatment relative to placebo. We further provide quality of life (QOL) outcome measures showing commensurate improvement in seropositive patients based on dose of latiglutenase. It is worth noting that the 3rd Gastroenterology Regulatory Endpoints and Advancement of Therapeutics (GREAT-3) conference sponsored by the US Food and Drug Administration (FDA) in 2015 specifically cited the need to develop treatments that address symptom suffering due to accidental gluten ingestion.[18]

2 | METHODS

2.1 | Clinical study design and subjects

The ALV003-1221 clinical trial (www.clinicaltrials.gov, NCT01917630) was a multi-centre, multinational, randomised, double-blind, placebo-controlled, dose-ranging study in symptomatic, established patients with CD. Details of the trial are reported elsewhere.[14,15] The symptoms of each subject were recorded for a 4-week baseline period followed by an eligibility and randomisation period (2-4 weeks) during which patients underwent serological and endoscopic analysis. The main criterion for randomisation in the study was histological evidence for active disease, as judged by a villus height:crypt depth ratio (Vh:Cd) ≤2.0. Both seropositive and seronegative CD patients were enrolled and stratified in this study. Qualified subjects entered into a 12-week study period during which either a placebo or a defined dose of latiglutenase (100, 300, 450, 600, 900 mg) was administered orally TID. The patient populations for the data presented here were for seropositive: PBO (n = 54), 600 mg (n = 35), 900 mg (n = 14) and for seronegative: PBO (n = 68), 600 mg (n = 45), 900 mg (n = 22). About 20% of these patients were invited to continue for another 12 weeks; however, we do not use that data in this paper because the population of seropositive patients across the different doses was too small to draw any statistical conclusions. Each participant gave informed consent. All biological samples were coded to maintain blinding, and all investigators performing sample analysis were unaware of the patients' diagnostic status or the study results.

2.2 | Celiac Disease Symptom Diary (CDSD©)

The CDSD is a patient reported outcome (PRO) instrument that consists of a daily diary recorded across 7-day periods that assesses common celiac symptoms (abdominal pain, bloating, tiredness, nausea, diarrhea and constipation). Patients were instructed to complete the CDSD diary each evening recording the presence or absence of individual symptoms occurring over the prior 24-hour period. If a given symptom was present on a given day, follow-up questions were asked to establish the severity of each event. Further detail regarding the CDSD and how it is administered and scored are provided elsewhere.[15,19,20] Briefly, for all symptoms except constipation each patient's daily severity score is normalised from 0 to 10 where 0 represents no symptom. The weekly score therefore ranges from 0 to 70. The frequency value is the number of nonzero events, irrespective of severity. A nonstool

composite severity score, consisting of all symptoms besides diarrhoea and constipation, was also computed. Constipation requires several days of data and is not amenable to a daily score is not recorded other than to measure the number of complete spontaneous bowel movements (CSBMs) per day. A constipation event is defined when less than three bowel events occur for the week. The severity of constipation is then calculated from the number of bowel movements for the week and ranges from 0 to 70. While we did not formally measure of constipation frequency; this was reported by convention by the number of bowel movements per week, however, in the following we will refer to this as "constipation frequency" for consistency with other measures.

2.3 | Impact of Celiac Disease Symptoms Questionnaire (ICDSQ)

The ICDSQ© was used to assess the impact of patients' celiac symptoms over the previous week at Day 1, Week 6 and Week 12. This was extended to Week 18 and Week 24 for the patients who volunteered to continue, but we do not include those data due to statistics with low n. The questionnaire was comprised of 14 items with four domains: Daily Activities (four items), Social Activities (three items), Emotional Well-being (five items) and Physical Functioning (two items). Each item had five response options ranging from "not at all" to "completely". Each domain was individually scored and an overall impact score was also calculated giving equal weight to each domain.

2.4 | Patient Global Impression-Symptoms (PGI-S)

The PGI-S assessed change over time in the severity of symptoms and impact of symptoms on the same visit schedule as for the ICDSQ. Patients were first asked patients to rate their symptom severity over the previous seven days on a 6-point rating scale from "no" to "very severe" symptoms in the PGI-S. For those patients reporting symptoms, the second PGI-S item asked patients to rate how much their celiac symptoms had a negative impact on their Daily Activities, Social Activities, Emotional Wellbeing and Physical functioning using a 5-point rating scale from 'not at all' to 'completely'.

2.5 | SF-12 v2® Health Survey

The SF-12 v2 Health Survey, a shorter version of the SF-36 Health Survey, asked patients to answer 12 questions that measure physical and mental health on the same visit schedule as for the ICDSQ and PGI-S.

2.6 | Symptom and QOL statistical analysis

The improvement value at each dose (I_{dose}) for each symptom and QOL was quantified using the following equation:

$$I_{dose} = [(\Delta B_{dose}/B_{dose}) - (\Delta B_{PBO}/B_{PBO})]/(1 - (\Delta B_{PBO}/B_{PBO})) \quad (1)$$

where B_{dose} is the baseline value (ie, the score of a particular outcome measure in the week prior to the Day 1 visit), and ΔB_{dose} is the change in baseline value for a particular dose in week 6 or week 12 of drug dosing. The subscript PBO represents the placebo dose population. The $(1 - (\Delta B_{PBO}/B_{PBO}))$ term in the denominator accounts for the improvement in a symptom or QOL measure due to latiglutenase activity relative to the placebo effect; as a result, I_{dose} can assume values between 0% (corresponding to the placebo effect) and 100% (full recovery in symptom or QOL outcome). *P*-values for $\Delta B_{dose}/B_{dose}$ (including dose = PBO) and $(\Delta B_{dose}/B_{dose}) - (\Delta B_{PBO}/B_{PBO})$ were stratified by serostatus and calculated by analysis of covariance (ANCOVA) and were not adjusted for multiplicity.

2.7 | Serum testing

The levels of anti-transglutaminase 2 (TG2) IgA and IgA and IgG antibodies to deamidated gliadin peptides (DGP) were measured by enzyme linked immunosorbent assays (ELISA). The trial results were stratified by serologic status as either negative or positive, with positive defined as above the normal range for any of the three serology assays.

3 | RESULTS

3.1 | Effect of latiglutenase dose on symptom severity and frequency

To estimate the extent to which latiglutenase dosing improved the frequency and severity of these symptoms above and beyond the placebo effect, the data collected at the two highest drug doses (600 and 900 mg) were analysed according to Equation 1, both in week 6 and week 12 of the study. The results reported in Table 1 underscore both a dose and a duration dependence of the symptomatic benefit due to latiglutenase in seropositive patients. The symptom domains showing the greatest benefit from latiglutenase are abdominal pain, bloating, tiredness and constipation (as measured by the complete spontaneous bowel movements, CSBM). The severity improvements relative to placebo were 58%, 44%, 21% and 104%, respectively, for abdominal pain, bloating, tiredness and constipation for the 900 mg dose level for week 12 (end of main trial). (The >100% RIS for constipation is an artefact of the PBO effect being <0%, Table S1). The *P*-values for these relative to placebo were 0.038, 0.023, 0.164 and 0.049, respectively. The respective values for the composite of 600 mg and 900 mg were 0.008, 0.007, 0.009 and 0.044 (previously reported).[15] Symptom frequency also showed meaningful improvement. Similar trends were observed for week 6, but at approximately 80% the improvement of the week 12 results. It should be noted that these significant dose-dependent results were observed despite a considerable trial (Hawthorne)/placebo effect as described in the Supporting Information (Table S1).

Nausea and diarrhoea are components of the CDSD PRO tool and inexplicably these domains did not show significant benefit due to latiglutenase. However, within measurement uncertainty, they did

TABLE 1 Symptom improvements for seropositive patients treated with 600 and 900 mg latiglutenase. Data were analysed according to Equation 1 for weeks 6 and 12 of the study.

	Abdominal Pain				Bloating				Tiredness				Constipation (CSBM)			
	Severity (%)	*P*-value	Freq. (%)	*P*-value	Severity (%)	*P*-value	Freq. (%)	*P*-value	Severity (%)	*P*-value	Freq. (%)	*P*-value	Severity (%)	*P*-value	Freq. (%)	*P*-value
Week 6 (900 mg)																
Seropositive	33.9	.165	13.9	.445	41.6	.021*	12.2	.364	24.9	.070	19.1	.074	23.2	.487	23.0	.230
Seronegative	3.4	.946	5.6	.771	4.5	.817	10.8	.364	9.6	.451	6.8	.422	−41.9	.675	−2.7	.785
Total	16.9	.314	8.8	.478	19.8	.094	10.1	.267	16.1	.075	11.6	.072	−11.8	.928	6.8	.599
Week 12 (900 mg)																
Seropositive	57.5	.038*	38.9	.034*	44.1	.023*	19.0	.189	20.6	.164	9.1	.391	104.4	.049*	37.7	.036*
Seronegative	−38.7	.075	−12.2	.263	2.7	.929	13.7	.402	15.4	.264	16.5	.092	−22.1	.824	3.5	.779
Total	5.6	.943	7.4	.625	18.5	.196	14.7	.170	17.2	.081	13.4	.068	36.0	.262	17.2	.115
Week 6 (600 mg)																
Seropositive	24.7	.165	17.1	.141	29.3	.040*	10.1	.317	23.4	.028*	16.7	.038*	46.9	.197	−12.1	.778
Seronegative	4.3	.927	−2.9	.633	2.9	.923	4.7	.712	−13.5	.125	−14.3	.049*	−76.7	.949	−5.4	>.999
Total	13.5	.301	5.7	.555	14.2	.160	6.4	.404	2.9	.604	−0.1	.926	4.5	.505	−8.3	.866
Week 12 (600 mg)																
Seropositive	48.0	.026*	28.3	.039*	31.1	.040*	6.3	.521	36.9	.002*	22.0	.012*	43.0	.252	−9.9	.790
Seronegative	−10.0	.532	−9.7	.303	11.4	.552	5.2	.769	−2.6	.742	−6.8	.403	−41.8	.726	2.1	.534
Total	17.9	.282	6.7	.551	19.6	.086	5.4	.542	14.8	.059	6.4	.280	8.7	.324	−3.4	.793

*$P \leq .05$.

not show any worsening either. Unexpectedly, seronegative patients showed insignificant benefit from latiglutenase. This was striking as there were no other properties, such as baseline severity and frequency of symptoms nor magnitude of the trial/placebo effect that differentiated seropositive from seronegative patients. We speculate on possible explanations for this observation in the Discussion section.

As previously reported, a dose-dependent effect of latiglutenase was observed on the severity and frequency of abdominal pain, bloating, tiredness and constipation (CSBM) for seropositive patients, but not for seronegative patients in both week 6 and week 12 of the study.[15] Although the ALV001-1221 trial was not powered for symptoms, the trend with dose is clearly evident in Table 1. For the Overall Non-Stool GI Specific Severity Score, notable LS-mean differences from PBO in change from Baseline were observed among seropositive patients for 600 – PBO, 900 – PBO, and (600 + 900) – PBO at Week 6 and 12 as tabulated in Table 2. All cases show $P < .05$ values. For the comparable table for seronegative patients (not shown) all cases show $P > .28$ values. Figure 1 shows that these seropositive trends follow a dose dependence.

Another noteworthy observation was that the magnitude of RIS for the 600 and 900 mg seropositive latiglutenase arms was greater for those patients experiencing greater (baseline) symptom severity as shown in Figure 2 for abdominal pain and bloating for 900 mg patients. A similar but less distinct trend was observed for tiredness as well as for all symptoms in the 600 mg arm. Finally, segmenting the seropositive populations according to baseline severity did not show any significant efficacy of latiglutenase for nausea or diarrhoea nor for any symptom in seronegative patients.

3.2 | Symptom responder analysis

We now provide a responder analysis in which we define responders as patients who exceeded a specific RIS threshold in a symptom domain based on the severity and frequency scales. Figure 3 plots the percent of responders relative to PBO, PRB_{dose}, using an equation similar to Equation 1 and given by

$$PRP_{dose} = (PR_{dose} - PR_{PBO}) / (1 - PR_{PBO}) \quad (2)$$

In all cases and for weeks 6 and 12 a positive responder effect is observed relative to PBO (except for a null result for constipation severity). A similar responder analysis using the condition $I_{dose} \geq 50\%$ gave similar results to that for ≥30% (Figure S2).

3.3 | Daily symptom analysis

We further analysed the daily CDSD data to determine the frequency of symptom occurrences as a function of severity. This analysis provides additional detail and substantiates the results for dose-dependent symptom improvement and increased improvement for patients with greater baseline severity. These results are presented in the Supporting Information.

3.4 | QOL dependence on serostatus

In Figure S5 there is a very noticeable trend towards positive benefit for seropositive patients and nonpositive benefit for seronegative patients for individual components of the QOL instruments ICDSQ, PGI and SF12v2 on combined 600 and 900 mg dose that is consistent for weeks 6 and 12.

Table 3 and Figure 4 show results for the ICDSQ overall score for seropositive and seronegative patients for weeks 6 and 12. The overall score for the composite of 600 and 900 mg treated patients has $P = .022$ for week 6. Figure 4 plots the ΔB_{dose} values for the overall score and shows a distinct dose dependent QOL benefit for seropositive, but not for seronegative patients, although statistical significance is not met for week 12 due to the much greater PBO effect.

Tables 4 and 5 tabulate the change from baseline for the SF-12v2 QOL instrument for weeks 6 and 12 for the physical and mental

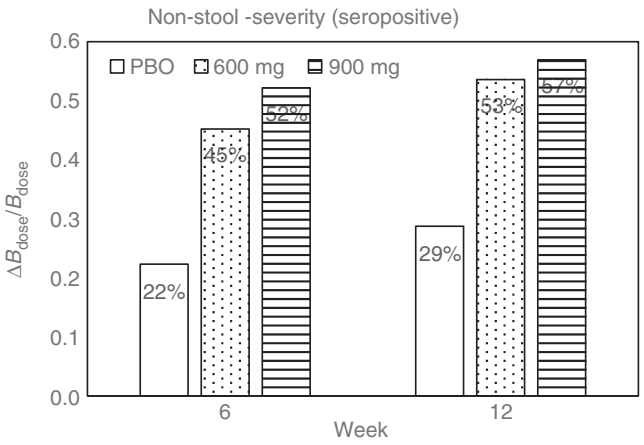

FIGURE 1 Change relative to baseline for weeks 6 and 12 in CDSD overall nonstool GI specific severity score among seropositive patients

TABLE 2 Analysis of covariance (ANCOVA) for change from baseline for weeks 6 and 12 in CDSD overall nonstool GI specific severity score among seropositive patients

	Week 6				Week 12			
Treatment Group	LS-Mean	SE	95% CI	*P* value	LS-Mean	SE	95% CI	*P* value
Placebo (PBO)	−8.47	3.09	−14.6, −2.38		−11.35	3.11	−17.5, −5.20	
600 – PBO	−10.98	4.97	−20.8, −1.17	.029	−11.08	4.93	−20.8, −1.34	.026
900 – PBO	−14.70	6.80	−28.1, −1.28	.032	−14.96	7.00	−28.8, −1.14	.034
(600 + 900) – PBO	−12.84	4.73	−22.2, −3.49	.007	−13.02	4.81	−22.5, −3.53	.007

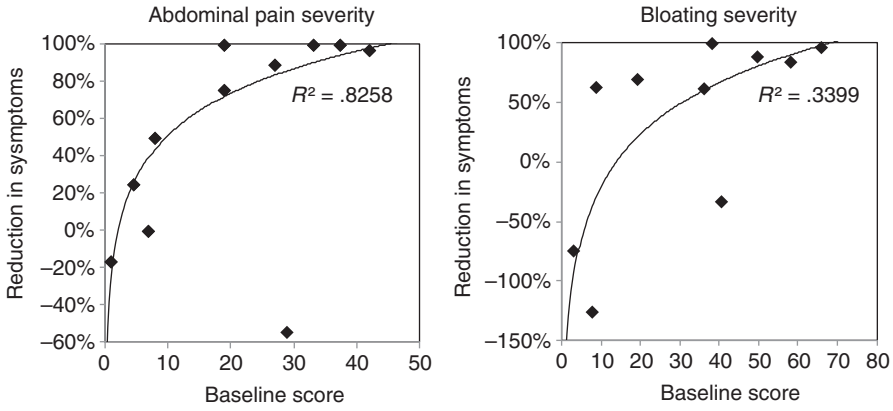

FIGURE 2 Dependence of reduction in abdominal pain and bloating severity as a function of baseline symptom scores for seropositive patients on 900 mg treatment. Baseline severity is on a weekly scale of 0-70. The trend fit and R^2 (logarithmic function) for abdominal pain excludes the outlier point at (*x*, *y*) = (28, −58%)

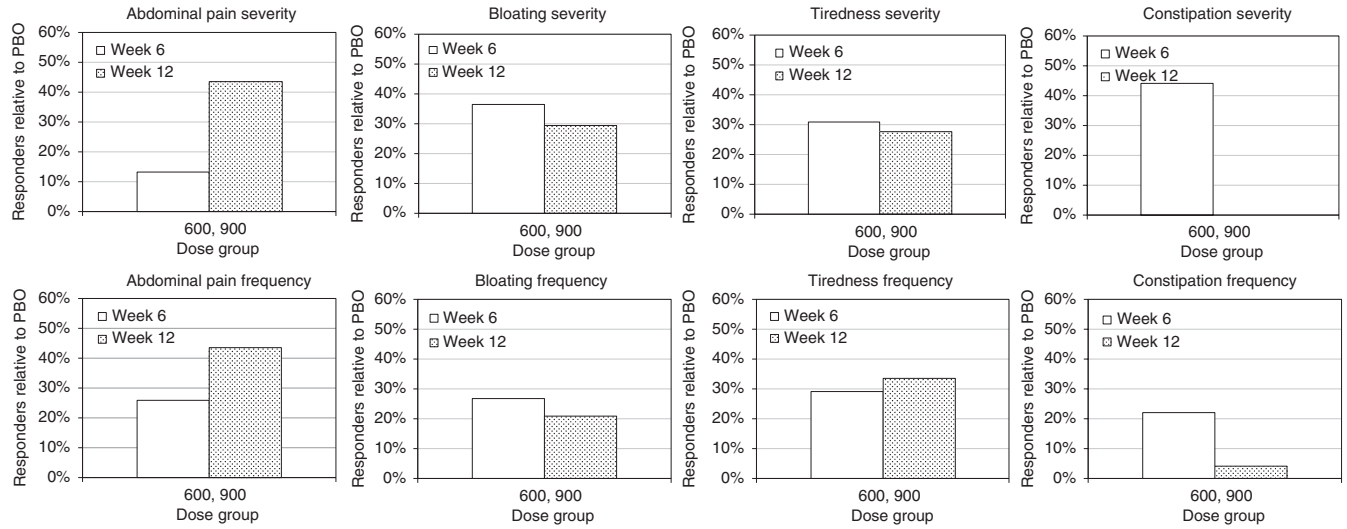

FIGURE 3 Responder analysis for ALV600 + 900 relative to PBO for a threshold of ≥30%

TABLE 3 Analysis of covariance (ANCOVA) for change from baseline for weeks 6 and 12 in ICDSQ overall score among seropositive patients

	Week 6				Week 12			
Treatment Group	**LS-Mean**	**SE**	**95% CI**	***P* value**	**LS-Mean**	**SE**	**95% CI**	***P* value**
Placebo (PBO)	−1.45	0.35	−2.13, −0.76		−2.36	0.35	−3.04, −1.67	
600 − PBO	−1.08	0.56	−2.19, 0.02	.055	−0.32	0.56	−1.43, 0.78	.565
900 − PBO	−1.35	0.74	−2.81, 0.11	.070	−0.59	0.75	−2.08, 0.89	.433
(600 + 900) − PBO	−1.22	0.52	−2.25, −0.18	.022	−0.46	0.53	−1.50, 0.59	.388

component scores, respectively. These results also show a statistically significant improvement in these components for seropositive patients in the 600 and 900 mg dose. Interestingly both measure decrease in going from week 6 to week 12, a trend also observed by the ICDSQ instrument. This appears to be due less to a decline in QOL improvement and more to an increased placebo effect for week 12.

4 | DISCUSSION

There is an urgent need for nondietary therapies for celiac disease. The ALV003-1221 "real-world" trial attempted to show mucosal healing for the treatment arms as a corollary to the successful ALV003-1021 "gluten-challenge" trial that showed protection of the mucosa for the treatment arm. The former trial, however, did not demonstrate significant mucosal healing for the latiglutenase arms relative to the placebo arm; instead all arms improved comparably. It was clear that the patient population improved their GFDs while on the trial accounting for improvements in the villous height to crypt depth ratio (Vh:Cd) as well as a strong improvement in symptom and QOL assessments for the PBO arm. Equally clear was that there was still sufficient unintended gluten ingestion in at least some patients to cause frequent symptom responses that were attenuated by latiglutenase treatment. CD

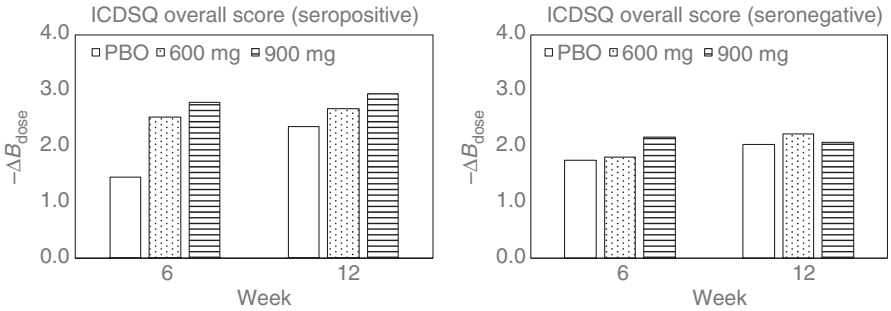

FIGURE 4 Negative change from baseline of ICDSQ overall score for weeks 6 and 12 among seropositive and seronegative patients. The data on the left plot are from Table 3

TABLE 4 Analysis of covariance (ANCOVA) for change from baseline for weeks 6 and 12 in SF-12v2: physical component score among seropositive patients (positive values denote improvement)

	Week 6				Week 12			
Treatment Group	**LS-Mean**	**SE**	**95% CI**	***P* value**	**LS-Mean**	**SE**	**95% CI**	***P* value**
Placebo (PBO)	3.2	3.0	−2.8, 9.2		6.5	2.9	0.8, 12.3	
600 − PBO	7.5	4.9	−2.2, 17.1	.129	3.8	4.7	−5.6, 13.1	.424
900 − PBO	14.2	6.5	1.3, 27.1	.031	−1.0	6.4	−13.7, 11.7	.876
(600 + 900) − PBO	10.8	4.6	1.7, 19.9	.020	1.4	4.5	−7.5, 10.3	.755

TABLE 5 Analysis of covariance (ANCOVA) for change from baseline for weeks 6 and 12 in SF-12v2: mental component score among seropositive patients (positive values denote improvement)

	Week 6				Week 12			
Treatment Group	**LS-Mean**	**SE**	**95% CI**	***P* value**	**LS-Mean**	**SE**	**95% CI**	***P* value**
Placebo (PBO)	0.8	2.3	−3.8, 5.4		8.2	2.1	3.9, 12.4	
600 − PBO	13.0	3.8	5.6, 20.4	<.001	6.8	3.5	−0.0, 13.6	.051
900 − PBO	7.9	5.0	−2.0, 17.8	.117	6.2	4.7	−3.0, 15.4	.184
(600 + 900) − PBO	10.4	3.5	3.5, 17.4	.004	6.5	3.3	0.1, 13.0	.048

patients even when increasing their diligence of a GFD still cannot avoid eliminating gluten entirely.

Although the ALV003-1221 trial was not powered for symptom improvement nor was this a primary endpoint, the serostatus stratified analysis showed surprisingly strong and statistically significant improvement for most CD-related symptom domains and almost all QOL component measurements for seropositive patients for high doses of latiglutenase. The principal trends were: (a) dose-dependent improvement in symptoms and QOL, (b) greater reduction in symptoms for more symptomatic patients, (c) consistent results for week 6 and 12 showing well developed symptom improvement by week 6 and small but continued improvement by week 12 for symptoms, and, (d) consistency of the severity and frequency reduction in symptoms in a responder analysis. Oddly whereas the symptom benefit maintained and even improved for week 12 vs week 6, the opposite trend was observed for the QOL components. This may be due to patients self-normalising from the previous reporting period such that instead of reporting a change from baseline they are biased toward reporting a change from the last reporting period. However, it is also evident in Figure 4 and Tables 3-5 that the placebo effect increased significantly from Week 6 to Week 12, which then diminished the change from baseline relative to placebo for the 600 and 900 mg patients.

A perplexing question is why the latiglutenase benefit is statistically significant for seropositive, but not seronegative patients. A potential explanation is that the symptoms observed in seronegative patients may not be predominantly due to gluten exposure or even to CD, but to other gastrointestinal ailments, such as functional GI syndromes that are common in CD patients.[21] A potential reason why this group would be enriched in the ALV003-1221 study is that the enrolment criteria set minimum symptom requirements in order to address the population of moderately to severely symptomatic patients. Another less likely explanation is that there may be a population of seronegative patients whose residual biopsy measured villous atrophy is not due to ongoing inadvertent gluten exposure.[22]

Another question is why latiglutenase reduces the symptoms of abdominal pain, bloating, tiredness and constipation, but not nausea and diarrhoea. We have no plausible explanations at this time; however, we do observe an increasing symptom benefit for all symptoms including nausea and diarrhoea with increasing time on a GFD, which we plan to explore further in future studies.

5 | CONCLUSION

The ALV003-1221 trial stratified the patient population with the intention to explore the impact of serostatus on responsiveness to the primary and secondary endpoints. While the study results did not demonstrate conclusive evidence of latiglutenase-induced mucosal healing as the primary endpoint, post analysis of the symptom and QOL data supporting secondary endpoints showed for seropositive patients statistically and clinically significant evidence of latiglutenase-induced reduction of several key symptoms associated with gluten ingestion in CD patients and a correlation to QOL improvement. The principal conclusions of this work include:

- A dose-dependent reduction in symptoms was observed for seropositive, but not seronegative patients for abdominal pain, bloating, tiredness and constipation (CSBM). Nausea and diarrhoea were not significantly responsive to latiglutenase.
- A trial (Hawthorne)/placebo effect was observed for the major symptoms and QOL assessments for seropositive and seronegative patients (RIS typically 20%-30%) further substantiating the trial effect for histology.
- Greater reduction in symptom severity and frequency was observed for more symptomatic patients in the week 6 and week 12 data.
- Patients experience symptom flares that reduce in frequency and severity under latiglutenase treatment.

The selection of subjects for a trial of a drug therapy that targets gluten will test most effectively if the subjects are likely to be exposed to gluten such as those that are seropositive.

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AUTHORSHIP

Guarantor of the article: Jack A. Syage, PhD takes responsibility as guarantor of the article.

Author contributions: JAM, PHRG, DCA, CK, JAS: performed the research. JAS, DCA, CK: collected and analysed the data. JAS, CK, JAM: designed the research study and wrote the paper. JAS, JAM, JASV, PHRG, DCA, CK: contributed to the design of the study.

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REFERENCES

1. Lohi S, Mustalahti K, Kaukinen K, et al. Increasing prevalence of celiac disease over time. *Aliment Pharmacol Ther*. 2007;26:1217-1225.
2. Schuppan D, Junker Y, Barisani D. Celiac disease: from pathogenesis to novel therapies. *Gastroenterology*. 2009;137:1912-1933.
3. Rubio-Tapia A, Murray JA. Celiac disease. *Curr Opin Gastroenterol*. 2010;26:116-122.
4. 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;107:201-207.
5. Lerner BA, Phan Vo LT, Yates S, Rundle AG, Green P, Lebwohl B. Detection of gluten in gluten-free labeled restaurant food: analysis of crowd-sourced data. *Am J Gastroenterol*. 2019;114:792-797.
6. Kelly CP, Bai JC, Liu E, Leffler DA. Celiac disease: clinical spectrum and management. *Gastroenterol*. 2015;148:1175-1186.
7. Green P, Lebwohl B, Greywoode R. Celiac disease. *J Allergy Clin Immunol*. 2015;135:1099-1106.
8. Guandalini S, Tundia N, Thakkar R, Macaulay D, Essenmacher K, Fuldcore M. Direct cost in patients with celiac disease in the U.S.A.: a retrospective claims analysis. *Dig Dis Sci*. 2016;10:2823-2830.
9. Gottlieb K, Dawson J, Hussain F, Murray JA. Development of drugs for celiac disease: review of endpoints for Phase 2 and 3 trials. *Gastroenterol Rep*. 2015;3(2):91-102.
10. Lähdeaho M-L, Kaukinen K, Laurila K, et al. Glutenase ALV003 attenuates gluten-induced mucosal injury in patients with celiac disease. *Gastroenterology*. 2014;146:1649-1658.
11. Wolf C, Siegel JB, Tinberg C, et al. Engineering of kuma030: a gliadin peptidase that rapidly degrades immunogenic gliadin peptides in gastric conditions. *J Am Chem Soc*. 2015;137:13106-13113.
12. Leffler DA, Kelly CP, Green P, et al. Larazotide acetate for persistent symptoms of celiac disease despite a gluten-free diet: a randomized controlled trial. *Gastroenterology*. 2015;148:1311-1319.
13. Daveson A, Ee HC, Andrews JM, et al. Epitope-specific immunotherapy targeting CD4-positive T cells in celiac disease: safety, pharmacokinetics, and effects on intestinal histology and plasma cytokines with escalating dose regimens of Nexvax2 in a randomized, double-blind, placebo-controlled phase 1 study. *EBioMedicine*. 2017;26:78-90.
14. Murray JA, Kelly CP, Green PH, et al. No difference between latiglutenase and placebo in reducing villous atrophy or improving symptoms in patients with symptomatic celiac disease. *Gastroenterology*. 2017;152:787-798.
15. Syage JA, Murray JA, Green P, Khosla C. Latiglutenase improves symptoms in seropositive celiac disease patients while on a gluten-free diet. *Dig Dis Sci*. 2017;62:2428-2432.

16. Shan L, Molberg O, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. *Science*. 2002;297:2275-2279.
17. Gass J, Bethune MT, Siegel M, Spencer A, Khosla C. Combination enzyme therapy for gastric digestion of dietary gluten in celiac sprue patients. *Gastroenterology*. 2007;133:472-480.
18. 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;151:407-411.
19. Leffler DA, Acaster S, Gallop K, Dennis M, Kelly CP, Adelman DC. A novel patient-derived conceptual model of the impact of celiac disease in adults: implications for patient-reported outcome and health-related quality-of-life instrument development. *Value Health*. 2017;20:637-643.
20. Hindryckx P, Leveseque BG, Holvoet T, et al. Disease activity indices in celiac disease: systematic review and recommendations for clinical trials. *Gut*. 2018;67:61-69.
21. Sainsbury A, Sanders DS, Ford AC. Prevalence of irritable bowel syndrome-type symptoms in patients with celiac disease: a meta-analysis. *Clin Gastroenterol Hepatol*. 2013;11:359-365.
22. Pallav K, Leffler DA, Tariq S, et al. Noncoeliac enteropathy: the differential diagnosis of villous atrophy in contemporary clinical practice. *Aliment Pharm Ther*. 2011;35:380-390.

SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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