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Beyond the gluten-free diet: Innovations in celiac disease therapeutics

Massironi S *et al.* Emerging therapies in CD management

Abstract

Celiac disease (CD) is an autoimmune disorder exacerbated by the ingestion of gluten in genetically susceptible individuals, leading to intestinal inflammation and damage. This chronic disease affects approximately 1% of the world's population and is a growing health challenge due to its increasing prevalence. The development of CD is a complex interaction between genetic predispositions and environmental factors, especially gluten, culminating in a dysregulated immune response. The only effective treatment at present is a strict, lifelong gluten-free diet. However, adherence to this diet is challenging and often incomplete, so research into alternative therapies has intensified. Recent advances in CD's molecular and immunologic understanding have spearheaded the development of novel pharmacologic strategies that should provide more effective and manageable treatment options. This review examines the latest innovations in CD therapies. The focus is on drugs in advanced clinical phases and targeting specific signaling pathways critical to the disease's pathogenesis. We discuss both quantitative strategies, such as enzymatic degradation of gluten, and qualitative approaches, including immunomodulation and induction of gluten tolerance. Innovative treatments currently under investigation include transglutaminase inhibitors, which prevent the modification of gluten peptides, and nanoparticle-based therapies to recalibrate the immune response. These new therapies not only promise to improve patient outcomes, but are also expected to improve quality of life by reducing the burden of dietary restrictions. The integration of these new therapies could revolutionize the treatment of CD and shift the paradigm from strict dietary restrictions to a more flexible and patient-friendly therapeutic approach. This review provides a comprehensive overview of the future prospects of CD treatment and emphasizes the importance of continued research and multidisciplinary collaboration to integrate these advances into standard clinical practice.

Key Words: Celiac disease; Gluten tolerance; Enzymatic degradation; Therapeutic advances; Transglutaminase inhibitors; Tight junction modulators

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Core Tip: The landscape of celiac disease treatment is evolving beyond the traditional gluten-free diet due to the challenges of strict adherence to the diet and incomplete resolution of symptoms. This review highlights emerging therapeutic strategies, including gluten sequestration and degradation, gluten tolerance induction, tight junction modulators, transglutaminase inhibitors, lymphocyte trafficking and homing inhibitors. These novel therapies, which target specific molecular and immune signaling pathways, promise to improve patient outcomes and quality of life by reducing dietary restrictions and addressing persistent inflammation and symptoms. Further research and multidisciplinary collaboration are critical to integrate these advances into standard clinical practice.

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INTRODUCTION

Celiac disease (CD), a chronic autoimmune small bowel enteropathy triggered by gluten ingestion in genetically predisposed individuals[1], affects approximately 1% of the global population, with an increasing incidence that has been detected worldwide[2,3]. The pathogenesis of CD involves a complex interplay between genetic predisposition and environmental factors, primarily gluten, which leads to intestinal inflammation and villous atrophy. ⁵ Currently, the only effective treatment for CD is a strict, lifelong gluten-free diet (GFD), which can be challenging to adhere to, and may not fully prevent the inflammatory responses and associated complications. Recent advances in understanding the molecular and immunological aspects of CD have opened new avenues for therapeutic interventions.

This review discussed the latest advances in the development of novel therapeutic approaches in CD, summarizing drug that are currently in advanced phases of clinical evaluation, targeting specific signaling pathways involved in the pathogenesis of the

disease. We will also discuss the potential of these drugs to change the treatment landscape for CD by offering alternatives to the dietary approach. By analyzing recent clinical trials and new research findings, we aimed to provide a comprehensive overview of the future prospects of CD treatment and how these new drugs could improve patient outcomes and quality of life.

CURRENT CHALLENGES IN CD MANAGEMENT

Gluten refers to a subgroup of wheat proteins, which comprises monomeric water-soluble gliadins and multimeric water-insoluble glutenins. It also includes, in sensum latum, secalin, hordein and avenins, which are found in rye, barley, and oats, respectively[4]. Due to its viscoelastic properties, gluten is an important and ubiquitous ingredient in the food industry. Apart from the obvious foods, *i.e.*, bread or pasta, it is also found in “unlikely” ones, such as soups, sauces, yogurt and frozen foods; in addition, it can be hidden in toothpaste and lipsticks, making strict adherence to the GFD very difficult[5].

Self-reported adherence to GFD ranges from 42% to 91% in adults and is about 59% in children, depending on age at diagnosis, ethnicity, cognitive, emotional and socio-cultural influences, membership of an advocacy group and regular dietetic follow-up[6,7]. It must be underlined, however, that even patients that are following a strict GFD can ingest gluten; infact, according to some studies, up to 70%-80% of adherent patients present gluten contamination[8,9], with an average gluten exposure of around 150 mg/day, which is much higher than the considered safe amount[10-12]. Actually, many products can be contaminated with gluten during harvesting, processing, packaging and cooking; in addition it could be difficult to control for gluten contamination while eating at restaurants, or using packaged food[13,14].

GFD is also burdened by some other problems. First of all, even if popularity has recently improved this matter, gluten-free products can be more expensive and difficult to find than gluten-containing ones[15]. Secondly, gluten-free products, to be more tasty, are often high in fat and sugar content and low in fibers, vitamins, and minerals

increasing, on one hand, the risk of obesity and metabolic syndrome and, on the other, the risk of deficiencies[16,17]. Additionally, there is a theoretical risk for mycotoxin exposure from corn and arsenic exposure from rice in those who restrict their diet to only a few carbohydrate sources[18,19]. Moreover, adherence to GFD can have negative effects on quality of life, leading to isolation, anxiety, depression, psychological distress and maladaptive food attitudes and behaviors[20-22]. Finally, despite strictly GFD, up to 30% of patients report persistent symptoms[23], about 30%-60% of adults will not achieve histological recovery after one year on a strict GFD[24] and up to 0.5% of patients with CD will progress to refractory CD[25]. Because of all these limitations, there has been a growing interest in non-dietary treatment options in recent years, since the marketing of additional therapies could improve the response to GFD and reduce its social limitation.

NEW STRATEGIES TO TREAT CD

The therapeutic horizon for CD is expanding beyond strict adherence to a GFD, due to advances in our understanding of pathophysiology and the emergence of new pharmacologic interventions. Gluten includes two different types of proteins, namely gliadins and glutenins; the former are regarded as the main culprit in the pathogenesis of CD, and several studies have demonstrated that different portions of alpha-gliadin can trigger immunity. Indeed, amino acids 31-43 activate the innate immunity, whereas the 33-mer targets adaptive immunity[26]. Gliadins are characterized by repeated sequences of glutamines and prolamines which are not easily processed by human digestive enzymes. This lack of digestion is particularly evident for the 33-mer peptide in CD subjects[26], allowing this peptide to ¹¹cross the epithelial barrier, be deamidated by the enzyme tissue transglutaminase 2 and bind to HLA-DQ2/8 molecules on antigen-presenting cells triggering gluten-specific CD4 T cells. These cells, in turn, activate CD8 T cells that cause the small-intestinal mucosal injury; all these processes are mediated by cytokines, such as interferon- γ , tumour necrosis factor α , interleukin (IL)-2, IL-21, and IL-15[27].

The elucidation of the pathogenesis of CD has led to the identification of multiple possible therapeutic targets, enabling the development of innovative treatment strategies. These strategies can be broadly classified into quantitative approaches, which aim to reduce the gluten load that triggers the immune response, and qualitative approaches, which aim to modulate the immune system and promote gluten tolerance. Quantitative strategies are diverse (Table 1). They include the use of exogenous peptidases to enzymatically digest gluten into non-immunogenic fragments, the sequestration of gluten peptides in the intestinal lumen to prevent their interaction with the mucosal immune system, and the reduction of intestinal permeability to prevent the translocation of immunogenic peptides. Each of these strategies aims to mitigate the antigenic stimulus underlying the pathophysiologic response in CD.

On the other hand, the qualitative approaches encompass a spectrum of modalities that alter the immune system's engagement with gluten. These include the inhibition of tissue transglutaminase, which plays a crucial role in the post-translational modification of gluten peptides, thereby reducing the formation of highly immunogenic complexes. In addition, the modulation of lymphocyte migration and homing offers an opportunity to prevent the recruitment and retention of inflammatory cells in the intestinal mucosa.

New research is also addressing the potential of desensitization to gluten through advanced biotechnological methods, such as nanoparticles engineered for targeted gliadin presentation, conjugation of gluten peptides to erythrocyte membranes, and therapeutic vaccines aimed at recalibrating the immune response. In addition, the paradigm of using helminth infestation to exploit natural pathways of immune regulation represents a novel and intriguing avenue of investigation.

EMERGING THERAPIES FROM PHASE-2 TRIALS

The pursuit of novel therapeutics in the treatment of CD has led to the initiation and progression of multiple phase 2 clinical trials.

Gluten sequestration and degradation

Gluten sequestration and degradation strategies, with a focus on enzymatic approaches, have been extensively investigated to mitigate the effects of gluten exposure in CD. These therapeutic interventions include several notable studies (Table 1). As regards gluten sequestration, BL-7010 is a high molecular weight, non-absorbable polymer (poly(hydroxyethyl methacrylate-co-styrene sulfonate, P(HEMA-co-SS) with a high affinity for gliadin[28], thus able to prevent the absorption of immunogenic and cytotoxic peptides. In fact, by targeting these peptides, BL-7010 could play a crucial role in reducing the inflammatory and immunogenic responses characteristic of CD. *In vitro* studies have demonstrated that BL-7010 is effective in decreasing gliadin/gluten-induced damage in cell cultures[29], these data have also been confirmed in a mouse model expressing the HLA-HCD4/DQ8 sensitized to gluten sensitization[28-30]. This drug has been assessed in NCT01990885, a randomized, double-blind study designed to evaluate its safety for single escalating doses as well as repeated administrations in well-controlled celiac patients. Although the trial took place about ten years ago, these results have not been published yet.

Another novel approach is the use of specific antibodies, such as avian immunoglobulin (IgY). IgY antibodies are obtained from the egg yolks of superimmunized laying hens. These antibodies are natural products with minimal toxicity, except for people with an egg allergy. They also offer a cost-effective and hygienic method of producing therapeutic agents. When the IgY antibody is formulated in capsules, it is referred to as AGY. The idea is to use these antibodies to capture gliadin peptides present in food; the trial NCT01765647 enrolled 10 patients to evaluate the potential of AGY to relieve CD symptoms and potentially reduce the burden of strict dietary control. However, it is noted that the results of this study were too weak to draw definitive conclusions. Study NCT03707730 is a randomized, double-blind, placebo-controlled, crossover study that evaluates the safety and efficacy of AGY in patients with CD on GFD. The study has completed the enrolment of 169 patients, but the data are not currently available. As mentioned before, the aminoacid composition of gluten in general, and of gliadins in particular, represents a difficult task for human

digestive enzymes. Thus, the quantitative reduction of food gluten content relies on enzymes derived from different sources (Table 1).

Latiglutenase is a combination of two glutenases, endoprotease B, isoform-2 (EP-B2) and *Sphingomonas capsulata* prolyl endopeptidase (SC-PEP). EP-B2 is active at low pH and has specificity for the QXP sequence, abundant both in the 31-43 as well as in the 33-mer peptides[31]. SC-PEP is a proline-specific endoprotease (PEP) which attacks the carboxy end of the gliadin peptides. The two enzymes can thus act together, with EP-B2 cutting the 33-mer peptides into smaller fragments and PEP digesting their proline-glutamine links[32]. Latiglutenase (ALV003) has been investigated in various studies. The phase 2b study NCT01917630 examined the effects of different doses of ALV003 over 12 weeks on the small intestinal mucosa and symptoms in patients with CD. Results showed no significant differences in the primary endpoint - villous height to crypt depth (Vh:Cd) ratio - nor in secondary endpoints like intraepithelial lymphocyte counts and serologic markers between the latiglutenase and placebo groups. A *post hoc* analysis[33] in a subgroup of patients still positive for autoantibodies indicated a dose-dependent reduction in symptom severity, especially at the highest dose (900 mg), suggesting potential benefits for seropositive patients.

Two other studies, *i.e.*, NCT01255696 and NCT00959114, investigated the efficacy, safety, and tolerability of ALV003 in patients with well-controlled CD. In NCT00959114, patients receiving ALV003 showed no significant mucosal damage after gluten challenge (2 g of bread crumbs) in contrast to the placebo group, which showed signs of mucosal deterioration. Morphological changes and the number of CD3+ intraepithelial lymphocytes showed significant differences between the groups, underlining the potential of ALV003 to mitigate gluten-induced intestinal damage[34]. Similarly, NCT01255696, a phase 2a, double-blind, placebo-controlled study, evaluated the safety, efficacy and tolerability of six weeks of treatment with ALV003 in patients with well-controlled CD. In summary, these studies suggest that while ALV003 does not significantly alter histologic or serologic markers of the disease in a broad cohort, it is

able to mitigate gluten-related mucosal damage in patients with CD and alleviate symptoms, particularly in seropositive individuals.

⁹ Latiglutenase was employed in other three studies: NCT03585478 was a phase 2 double-blind, placebo-controlled study assessing the efficacy and safety of a 1200 mg dose of IMGX003 in CD patients. Participants were exposed to 2 g of gluten daily for six weeks; the primary endpoint was the change in the Vh:Cd ratio, and results indicated a lower mean change in the Vh:Cd ratio and intraepithelial lymphocyte density for IMGX003 compared to placebo, alongside reduced symptom severity[35]. NCT04243551 is a phase 2b, multicenter, randomized, double-blind, placebo-controlled, crossover study involving symptomatic CD patients who had been on a GFD for at least one year prior to the study. The study has been completed and the results are currently awaited. NCT04839575 is ¹⁰ a prospective, double-blind, placebo-controlled, crossover study that investigated the efficacy and safety of latiglutenase treatment in type 1 diabetes patients with CD on a regular gluten exposure. It was terminated due to coronavirus disease 2019 disruptions and enrollment challenges.

TAK-062 is a computer-designed enzyme based on the bacterial kumamolisin-As, obtained from *Alicyclobacillus sendaiensis*; interestingly, the modifications enable this enzyme to target the proline-glutamine dipeptide. Its efficacy was assessed both *in vitro* and, in phase 1, *in vivo*, showing that it is able to degrade over 99% of gluten in complex meals[36]. These latter data are quite interesting since the assessment of gluten degradation was demonstrated analyzing the aspirate of the stomach content, thus in a physiological condition. NCT05353985 is ⁶ a phase 2, randomized, double-blind, placebo-controlled, dose-ranging study evaluating the efficacy and safety of TAK-062 in reducing CD-related symptoms and intestinal damage in CD patients attempting a GFD. The study includes two cohorts with different treatment groups receiving TAK-062 or placebo along with simulated inadvertent gluten exposure. The multi-center study, conducted across the United States, Canada, the United Kingdom and the European Union, includes adult and adolescent participants and is still ongoing.

The *Aspergillus niger*-derived prolyl endoprotease (AN-PEP), is able to cleave immunogenic gliadin peptides (behind proline residues) into smaller, non-immunogenic peptides of about eight amino acids. This enzyme is active between a pH of 2 and 8 and is resistant to pepsin, thus apt to be used to degrade gliadin ingested with food[37-39]. AN-PEP was investigated in NCT00810654[40] study that involved only 16 adults. AN-PEP was well tolerated and there were no serious adverse events or withdrawals. The efficacy phase showed no significant worsening of CD quality scores or antibody titers in patients who consumed gluten with either placebo or AN-PEP. Histologic and immunohistochemical evaluations also indicated stability in participants taking gluten with AN-PEP. The study concluded that AN-PEP was well tolerated, but the lack of clinical differences compared to placebo made it difficult to determine the effect of the enzyme. A double-blind, randomized, placebo-controlled trial employing commercial AN-PEP on patients on GFD has recently been published[41]. Although the patients in the treatment arm showed a reduction in symptoms, no significant difference in the level of gliadin immunogenic peptide in the stools was observed, data that could be explained by the relatively low levels detected in the run-in period. Different preparations of this enzyme are already available over the counter, but their ability to digest gluten peptides can be different and, in some cases, inferior to the pure enzyme[42]. However, CD patients should be warned of the potential risks of relying on these over-the-counter products, as their effectiveness may not be sufficient to prevent gluten-related damage.

Gluten tolerance

These therapies are designed to induce some form of tolerance to gluten in individuals with CD, possibly through immunomodulatory mechanisms or other pathways that reduce the pathological response to gluten. This approach doesn't necessarily involve the direct breakdown or sequestration of gluten but instead modifies the body's response to its presence.

The first-in-human phase 1 study (NCT04248855)[43], performed on celiac patients on GFD, evaluated the safety and tolerability of KAN-101, a synthetic liver-targeting glycopolymer that is conjugated to a synthetic deaminated peptide domain of wheat alpha gliadin designed to induce immune tolerance to gliadin. Due to the specifics of the employed peptide, this approach is reserved to individuals carrying the HLA DQ2.5 genotype, and the drug must be administered through the intravenous route. The mechanisms detected in preclinical studies through which KAN-101 could induce immunologic tolerance include selection of antigen-specific T cells, induction of anergy of antigen-specific T cells, and induction of regulatory T cells. The study demonstrated that KAN-101 (at increasing doses of 0.15 mg/kg, 0.3 mg/kg, 0.6 mg/kg, 1.2 mg/kg, and 1.5 mg/kg) exhibited an acceptable safety profile without any dose-limiting toxicities, and no maximum tolerated dose was identified. The rapid clearance of KAN-101 from the system and the absence of accumulation with repeated doses underscore the potential for chronic use.

Two studies focusing on KAN-101 are actively recruiting (Table 2). The first study, KAN-101-03 (NCT06001177) is a multicenter, double-blind, placebo-controlled phase 2a trial. Its primary objective is to examine the protective effects of KAN-101 against gluten-induced histological changes in the duodenum of adult participants with CD who adhere to a GFD. Additionally, the study aims to further evaluate the safety and tolerability profile of KAN-101. In parallel, study NCT05574010 adopts a two-part, multicenter phase 1b/2 design to evaluate the effects of KAN-101 in participants with CD on a GFD. Part A consists of an open-label, multiple ascending dose assessment to determine the safety, tolerability, and pharmacokinetics of KAN-101 in adults with histologically confirmed CD. Part B progresses to a double-blind, placebo-controlled format to characterize biomarker responses post-gluten challenge, alongside further safety, tolerability, and pharmacokinetic assessments.

The investigation of gluten tolerance in CD has led initiation of studies on TAK-101, which consists of gliadin encapsulated in negatively charged poly(DL-lactide-co-glycolic acid) nanoparticles (Table 2). These nanoparticles, intravenous injected, are taken up by

positive antigen-presenting cells localized in the liver and in the spleen; the presentation of gliadin to gliadin-specific T cells can induce tolerance through anergy and the activation of Treg cells. TAK-101's initial clinical assessment involved a phase 1 dose-escalation study (NCT03486990). The study's outcomes indicated that TAK-101 was well-tolerated, with no serious adverse events, clinically meaningful changes in vital signs or routine clinical laboratory evaluations, indicating an acceptable safety profile[36]. The double-blind, randomized, placebo-controlled phase 2a study (NCT03738475) was pivotal in evaluating the efficacy of TAK-101 in attenuating gluten-induced immune activation in CD. Thirty-three patients on GFD underwent a 14-day gluten challenge, with the primary endpoint being the change from baseline in circulating gliadin-specific interferon- γ -producing cells, which directly correlates with the pathophysiologic immune response in CD. TAK-101 administration resulted in an 88% reduction in interferon- γ -producing units compared to placebo, a statistically significant finding ($P = 0.006$) indicating a strong immunomodulatory effect. In addition, Vh: Cd ratio analysis revealed less deterioration in the TAK-101 group compared to placebo, although the difference did not reach statistical significance. TAK-101 also showed efficacy in modulating circulating $\alpha\beta\gamma$ +CD4+, $\alpha\beta\gamma$ +CD8+ and $\gamma\delta$ effector memory T cells, suggesting systemic immunomodulation[44]. Further investigation of TAK-101 is being conducted in a subsequent phase 2 study (NCT04530123), a randomized, double-blind, placebo-controlled and dose-ranging study. This study aims to evaluate the efficacy of TAK-101 in reducing gluten-related symptoms and immune activation in adult CD patients following a GFD and undergoing a gluten challenge, and its primary completion date was expected in May 2024.

Another drug aiming at inducing gluten-tolerance is TPM502, a mixture of nanoparticles carrying three peptides each consisting of two overlapping T cell epitopes that encompass the major gluten epitopes for HLA-DQ2.5. The ongoing NCT05660109 phase 2a study aims to evaluate its safety, tolerability, and pharmacodynamic effects

according to different doses, and its primary completion date was expected in May 2024 (Table 2).

Although the studies described above have provided encouraging data, among the drugs aiming at inducing tolerance it must also be quoted Nexvax2[®], a gluten peptide-based antigen-specific immunotherapy which aims to desensitize and make T cells unresponsive to gluten exposure. The first phase 1 clinical trial (NCT00879749) confirmed its bioactivity; however a following randomized clinical trial didn't show any advantage in preventing intestinal damage[27,45]. Furthermore, a phase 2 clinical trial (NCT03644069) evaluating its efficacy on patient-reported outcomes was terminated prematurely after an interim analysis because Nexvax2[®] was not able to reduce acute gluten-induced symptoms.

Tight junction modulators

Research into modulators of the tight junctions in CD has focused primarily on larazotide acetate, a synthetic octapeptide that reduces tight junctions' permeability by blocking zonulin receptors, thus designed to prevent the opening of the tight junctions in the intestinal epithelium and thereby reducing the passage of gluten peptides and the consequent immune activation[46]. Changes in zonulin levels are already detectable in the very early stages of CD and could serve as early biomarkers for the disease[47,48]. In addition to zonulin-dependent mechanisms, research has also identified zonulin-independent constitutional changes in intestinal permeability in CD patients and their first-degree relatives[47]. These inherent permeability alterations may contribute to the development and progression of CD[47,49].

However, several phase 2 studies have investigated the efficacy and safety of larazotide (Table 3). A phase 2b study (NCT00492960) included 184 patients on GFD[50]. Participants received larazotide acetate (1, 4 or 8 mg three times daily) or placebo along with 2.7 g of gluten daily for six weeks. Although no significant changes in the lactulose to mannitol ratio were detected, the researchers observed that the 1 mg dose of larazotide acetate significantly reduced gluten-induced symptoms, as well as

the increment in anti-tissue transglutaminase antibodies caused by gluten challenge. The study thus suggested that larazotide acetate may reduce gluten-induced immunoreactivity and symptoms in CD patients undergoing gluten challenge. In the study NCT00362856, a dose-ranging, placebo-controlled study, larazotide acetate limited gluten-induced worsening of gastrointestinal symptoms at lower doses, but not at higher doses. The study concluded that while larazotide acetate has the potential to prevent the severity of gluten-induced symptoms, its effects on intestinal permeability are unclear due to the variability of lactulose to mannitol[51]. Similarly, in a double-blind, placebo-controlled phase 2B study (NCT01396213), three doses of larazotide acetate were evaluated as an adjunct therapy to GFD in CD patients. The 0.5 mg dose of larazotide acetate significantly reduced symptoms compared to placebo. The study concluded that larazotide acetate 0.5 mg effectively reduced signs and symptoms in CD patients adhering to a GFD, representing a successful trial of a novel therapeutic agent targeting tight junction regulation[52]. Another study (NCT00620451) evaluated the efficacy of larazotide acetate in CD. This outpatient, randomized, double-blind study aimed to evaluate the efficacy of larazotide acetate in inducing remission in active CD, but did not provide specific results. Again, the study NCT00889473, an extension of study NCT00492960, aimed to evaluate the safety, tolerability and efficacy of larazotide acetate in a gluten challenge setting. Specific results were not presented.

Overall, these studies highlight the potential of larazotide acetate as a therapeutic agent for symptomatic relief in patients adhering to a GFD. Although results were mixed regarding its effects on intestinal permeability, its ability to alleviate gluten-related symptoms seemed to offer a promising avenue for improving the quality of life of people with CD. Thus, the findings from these phase 2 studies laid the groundwork for further exploration in phase 3 trials.

Transglutaminase inhibitors

Transglutaminase, modifying gluten peptides, is essential for gluten-induced T cell activation and the possibility to inhibit it has been widely studied. The more promising

molecule in this setting is ZED 1227, assessed by Schuppan *et al*[53] in a phase 2, double-blind, placebo-controlled trial. In this trial, 163 patients were randomly assigned to receive 10 mg of ZED 1227, 50 mg of ZED 1227, 100 mg of ZED 1227, or placebo during gluten challenge with a moderate amount (3 g) of daily gluten intake for 6 weeks. The primary endpoint was Vh:Cd ratio, as a marker of mucosal damage. The secondary endpoints included intraepithelial lymphocyte density, ¹³ the modified Marsh-Oberhuber classification and patient-reported outcomes measured by the Celiac Symptom Index and the Celiac Disease Questionnaire. ZED1227 significantly improved Vh:Cd ratio and attenuated intraepithelial lymphocyte density dose-dependently, whereas improved Celiac Symptom Index and the Celiac Disease Questionnaire independently to the dose[53]. Due to the activity of transglutaminase in several tissues, it was important to assess that the effect was limited to the intestine. For this reason, the same group assessed the loading of ZED 1227 in the biopsies of patients treated in the phase 2 study, showing the presence of the drug mostly in the epithelium (about 80%), with only about 20% present in the lamina propria. These data also prompted the authors to hypothesize that the drug exerts its effect mainly through an inhibition at the epithelial level[54].

Lymphocytes' trafficking and homing inhibitors

Strategies for immune modulation can include inhibition of lymphocyte proliferation, inhibition of lymphocyte trafficking and homing to the small bowel, and inhibition of the anti-inflammatory response (Table 4). CCX282-B is an orally administrated chemokine receptor-9 (CCR9) antagonist previously studied for the treatment of Crohn's disease, in which it generated contrasting results. CCR9 is expressed on circulating lymphocytes and is the key chemokine receptor determining intestinal homing[55,56]. The ligand of CCR9 is C-C chemokine ligand 25, which is expressed in the intestinal epithelium and is upregulated in the presence of inflammation[57]. A double-blind, randomized, placebo-controlled, phase 2 study (NCT00540657) evaluated its effectiveness in mitigating the effects of gluten ingestion in patients with CD. Ninety

patients were enrolled and half of them were treated by CCX282-B 250 mg twice daily for thirteen weeks. The primary outcome was the evaluation of the effect of CCX282-B compared to placebo on the Vh:Cd¹⁷ ratio of small intestinal biopsy specimens taken from subjects with CD, before and after gluten exposure. Secondary outcomes were the evaluation of small intestinal mucosal inflammation, celiac serology, and symptom scores. Although the study was completed in 2008, its results have never been published.

Another component necessary for the “gut-homing phenotype” is constituted by $\alpha 4\beta 7$ -integrin, belonging to a heterodimeric noncovalently bound transmembrane receptor family involved in cell-cell and cell-extracellular matrix interactions. This specific heterodimer is present in more than 90% of intestinal lymphocytes, and its main ligand is the mucosal addressin cell adhesion molecule, present in the gastrointestinal tract and associated lymphoid tissue[58,59]. For this reason, a phase 2 study (NCT02929316), aimed at evaluating if vedolizumab, a well-known monoclonal antibody against integrin $\alpha 4\beta 7$, approved for the treatment of ulcerative colitis and Crohn’s disease, could prevent small bowel atrophy in CD patients after gluten challenge. However, this study was stopped in October 2018, due to lack of enrolment. Another approach could be preventing lymphocyte proliferation, possibly focusing on intestinal ones reacting to gluten peptides. In 2021⁴ a phase 2a, double-blind, randomized, placebo-controlled study (NCT04806737) evaluated the efficacy and tolerability of a 14-day treatment with teriflunomide in 15 subjects with CD, undergoing a 3-day gluten challenge. Teriflunomide is an orally administrated drug currently approved for the treatment of multiple sclerosis. It inhibits *de novo* synthesis of pyrimidine, performing a cytostatic effect on lymphocyte proliferation[60]. Anyway, even in this case results are not available.

Lymphocytes involved in the pathogenesis of CD can be prompted to induce intestinal damage through the production of different mediators, including cytokines. Among them, a pivotal role has been recognized for IL-15, which can act on several cell types, including intraepithelial lymphocytes. A double-blind, phase 2a trial

(NCT02637141) investigated the effect of AMG-714, an anti-IL-15 monoclonal antibody, in patients with CD undergoing gluten challenge. In this study, 64 patients were randomly assigned to 150 mg AMG 714, 300 mg AMG 714, or placebo, administered by two subcutaneous injections every 2 weeks for 10 weeks. Patients without severe villous atrophy at baseline received also a gluten challenge. Duodenal biopsies were done at baseline and at the end of the study, in order to evaluate change in Vh: Cd ratio as primary outcome. Secondary outcomes included intraepithelial lymphocyte density, improvement in Marsh-Oberhuber score, changes in anti-transglutaminase and anti-deaminated gliadin peptide antibodies, number of bowel movements, percentage of diarrhea and changes in Gastrointestinal Symptom Rating Scale Score and in total Celiac Disease Gastrointestinal Symptom Rating Scale, questionnaires used to assess symptoms as diarrhea, indigestion, constipation, abdominal pain and reflux. The study demonstrated that Vh: Cd ratio was not significantly different between the groups of patients. However, changes in lymphocyte density and in symptoms suggest that further research of AMG 714 may be warranted in patients with non-responsive CD[61].

In fact, due to these encouraging results, the NCT02633020 trial evaluated the efficacy and safety of AMG 714 in patients with type II refractory CD. In this study 28 refractory CD patients were randomly assigned to 8 mg/kg AMG 714 or placebo intravenous infusion on day 0, day 7 and every 2 weeks for ten weeks. The primary outcome was to evaluate the reduction from baseline of aberrant intestinal intraepithelial lymphocytes, measured by flow cytometry after small intestinal biopsy collection. According to the study, there was no difference between the groups in terms of the primary endpoint of aberrant intraepithelial lymphocyte reduction from baseline, but there was a reduction of the number of patients with diarrhea[62].

Another ongoing phase 2b trial (NCT04424927) is evaluating the efficacy and safety in adult patients with non responder CD on a GFD of three dose regimens of PRV-015, which is also a monoclonal antibody against IL-15. This study is expected to be finished in August 2024. The ongoing trial NCT05636293 aims to establish safety and efficacy to prevent gluten-induced enteropathy and symptoms in CD patients of ritlecitinib, a

selective Janus kinase 3 (JAK3) inhibitor. JAK is a family of non-receptor tyrosine kinases, which include, in mammals, JAK1, JAK2, JAK3 and tyrosine kinase 2. Each protein has a kinase domain and binds cytokine receptors through amino-terminal domains. Upon binding of the ligand to cytokine receptors, JAKs are activated and phosphorylate the receptors, allowing the binding of the signal transducer and activator of transcription family members[63]. In addition to the inflammatory response, several studies have demonstrated that JAKs are essential for intestine differentiation and damage repair[64-66]. IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 depend on both JAK1 and JAK3 to elicit their intracellular effects, and JAK3 is expressed in immune cells as well as in intestinal epithelial cells. In the trial, participants are randomized to placebo or ritlecitinib 200 mg capsule once per day and are also taking 10 g gluten once per day, for a total of 21 days, decreasing to 5 g daily after day 3 of the study if not tolerated. The primary outcome is to evaluate changes in small bowel histology based on Vh:Cd ratio, while the secondary outcomes are focused on patients' reported outcomes. The study will finish in 2025.

Helminth infestation

According to the "hygiene hypothesis" reduction in the incidence of infectious diseases, especially of the helminth ones, can be responsible for the increasing prevalence of allergic and autoimmune diseases. In this scenario, arose studies about the possibility of suppressing the immunopathology induced by gluten and restoring tolerance in CD-inoculating patients with hookworms. NCT00671138 was a prospective, randomized, double-blinded, placebo-controlled phase 2 trial evaluating the safety, tolerability and immunological effects of *Necator Americanus* infection in subjects with CD in remission on GFD during gluten challenge. This trial enrolled 20 patients: Ten of them were inoculated with hookworm and compared with the other ten uninfected patients. Duodenal and rectal biopsies were performed before and after gluten challenge; blood samples were also collected in the same times. Mucosal damage, systemic inflammatory response, clinical response to gluten challenge, and mucosal inflammatory response

were not different in the two groups of patients before and after gluten challenge. So, even if hookworm infection was safe, it was not able to mitigate the small bowel damage induced by gluten[67]. On the other hand, a following phase 2 study (NCT01661933) aiming to establish the influence of hookworm infection in preventing intestinal damage and symptoms using escalating gluten challenges, suggested that it could promote immune regulation, provoking tolerance to gluten in CD. A more recent randomized, placebo-controlled phase 1 trial (NCT02754609) conducted on 54 patients concluded that hookworm infection does not restore tolerance to sustained moderate consumption of gluten, but it is associated with improved symptom scores after intermittent consumption of very low gluten doses[68]. However, considering the nature of the treatment it is difficult to imagine routine clinical use of hookworm infection in the management of CD.

PROGRESS IN PHASE 3 TRIALS

Larazotide acetate, a promising therapeutic agent for CD, has been the subject of extensive clinical research, culminating in its progression to phase 3 trials. The phase 3 trial of larazotide acetate (NCT03569007), also known as CedLara, represented a critical step in the drug's development and aimed to evaluate its efficacy and safety in alleviating symptoms in CD patients. Whereas larazotide resulted promising in phase 2 trials in alleviating gluten-related symptoms in CD patients adhering to a GFD, recent developments in the ongoing phase 3 trial have posed significant challenges. An independent statistical analysis showed that a substantial increase in the number of participants was required to achieve scientifically meaningful results. The additional need for patients was deemed too great, so the company overseeing the trial, concluded that it was not feasible to continue the trial under these conditions.

DISCUSSION

The current landscape of CD treatment is on the cusp of a paradigm shift. For decades, CD has been treated primarily dietary, with a strict GFD at its core. Although the GFD

is effective for many, it comes with significant challenges, including dietary restrictions, social and psychological distress, and the risk of accidental gluten exposure. Our review highlights the need for alternative therapeutic strategies that address these unmet needs in the treatment of CD (Figure 2). The development of non-dietary therapies, such as gluten sequestrants, transglutaminase inhibitors and lymphocyte trafficking inhibitors, represents a major advance. These new therapies offer the promise of reducing the burden of strict dietary adherence and improving the quality of life for CD patients. However, there are still some challenges. While phase 2 studies are promising, the efficacy and safety profiles of these therapies in broader patient populations need to be further validated in phase 3 studies. For instance, therapies such as larazotide acetate and ZED 1227 have shown the potential in mitigating gluten-induced symptoms and intestinal damage, but their long-term effects and side-effect profiles need to be studied more extensively. Moreover, CD is a heterogeneous disease and individual responses to these emerging therapies may vary. Personalized medicine approaches, potentially incorporating genetic, immunological and microbiological data, could play a critical role in optimizing treatment efficacy. As these therapies are tested in clinical trials, there is a need for confirmation in phase 3 trials for their integration into clinical practice. This integration will likely require multidisciplinary collaboration, including gastroenterologists, dietitians and patient education specialists. In addition, the role of these therapies in specific patient groups, such as patients with refractory CD or those at high risk of complications, needs to be investigated.

The advent of new therapies also brings with it ethical and social considerations. The autonomy of patients and their right to choose between dietary or pharmacological treatment must be respected. However, the development of new pharmacological treatments is costly and it is important to consider that resources should be prioritized where there is a clinical need. While respecting patient autonomy in choosing between dietary or pharmacologic treatment, it is critical to balance these options with the economic impact and practicality of making advanced treatments available to all patients who can benefit from them. Looking forward, the field of CD treatment is

ready for further discoveries and innovations. Future areas of research include the development of personalized treatment strategies, long-term safety studies of new drugs, and research into adjunctive therapies to improve quality of life. In addition, ongoing research into the pathophysiology of CD may reveal novel therapeutic targets.

CONCLUSION

The horizon of CD treatment is expanding beyond dietary treatment, giving hope for better outcomes for patients. However, the path from promising clinical trial results to practical, everyday treatments is complex and requires careful consideration of efficacy, safety, accessibility, and patient preference. Continued research, patient-centered care and collaborative clinical practice will be critical to making these emerging therapies a new standard in the treatment of CD.

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