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How Future Pharmacologic Therapies for Celiac Disease Will Complement the Gluten-Free Diet




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The only proven treatment for celiac disease is adherence to a strict, lifelong, gluten-free diet. However, complete dietary gluten avoidance is challenging and a substantial number of patients do not respond fully, clinically, or histologically, despite their best efforts. As celiac disease is common and its central pathophysiology is well elucidated, it has become attractive for drug development to address the limitations of dietary treatment. Most efforts address nonresponsive celiac disease, defined as continued symptoms and/or signs of disease activity despite a gluten-free diet, and the more severe forms of refractory celiac disease, types I and II. An increasing spectrum of therapeutic approaches target defined mechanisms in celiac disease pathogenesis and some have advanced to current phase 2 and 3 clinical studies. We discuss these approaches in terms of potential efficiency, practicability, safety, and need, as defined by patients, regulatory authorities, health care providers, and payors.

Keywords: Gluten Challenge; Clinical Trials; Immune Tolerance; Nonresponsive Celiac Disease.

Key features of celiac disease (CeD) are well understood.[1–4] Thus, certain gluten peptides (present in modern and ancient wheat, barley, and rye) that escape intestinal digestion can enter the small intestinal mucosa, where they are sensed by the mucosal immune system. Recognition by T cells requires their antigenic presentation on HLA-DQ2 or -DQ8, which are expressed prominently on myeloid (ie, monocytes, macrophages, and dendritic cells) and B cells. HLA-DQ2 or -DQ8 is a necessary but insufficient genetic precondition for CeD because approximately 30%–50% of most populations carry this predisposition, and CeD prevalence usually does not exceed 1%–2%, implicating other environmental (eg, nutritional and microbial), infectious, or genetic factors that trigger CeD.[5] Gluten peptide modification (deamidation) by the enzyme and CeD autoantigen, tissue transglutaminase 2 (TTG), occurs both at the level of the lamina propria and in the intestinal lumen.[6] Deamidation at specific residues increases the affinity of certain gluten peptides to the 9-amino acid binding pocket of the HLA-DQ2 or -DQ8.[7,8] This improved binding, a precondition for autoimmunity, markedly enhances the stimulation, expansion, and maintenance of gluten-specific $CD4^+$ T helper 1 cells. Continued supply of dietary gluten maintains the resultant small intestinal inflammatory response and enhances the small intestinal barrier defect that further increases gluten peptide influx into the mucosa. The gluten-specific $CD4^+$ T cells also help intestinal plasma cells produce antibodies to (deamidated) gluten peptides and to the autoantigen TTG.[9]

This gluten-specific T-cell response is necessary, but not sufficient, to promote tissue damage since patients with potential CeD have high anti-TTG autoantibodies but lack villous atrophy.[10] To develop overt CeD, full activation of cytotoxic $CD8^+$ intraepithelial lymphocytes (CT-IELs) is also required, characterized by up-regulation of activating natural killer–like receptors, down-regulation of inhibitory

Abbreviations used in this paper: **CeD, celiac disease; CT-IEL, cytotoxic $CD8^+$ intraepithelial lymphocyte; ER, enteric release; HLA, human leukocyte antigen; IEL, intraepithelial lymphocyte; IFN, interferon; IL, interleukin; NRCeD, nonresponsive celiac disease; PRO, patient-related outcome; RCeD, refractory celiac disease; RCeDI, type I refractory celiac disease; RCeDII, type II refractory celiac disease; TTG, tissue transglutaminase 2.**

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natural killer–like receptors, and expression of granzyme B and perforin, typically found in patients with untreated CeD. Increased levels of both interleukin (IL)-15 and IL-21 in the duodenal mucosa of untreated patients cooperatively promote the activation of CT-IELs by means of increasing their transcriptional, proliferative, and cytolytic activities.[11] The activation of CT-IELs also requires stimuli from the epithelial compartment (ie, increased expression of stress-induced molecules, such as major histocompatibility class I polypeptide–related sequence A or B and HLA-E, which serve as ligands for natural killer–like receptors.[1] The interaction between CT-IELs and stressed epithelial cells determines their cytotoxic lysis and leads to the tissue damage typically observed in patients with untreated CeD, characterized by intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy. Figure 1 presents a simplified overview of the key mechanisms of CeD.

Notably, the rigorous exclusion of gluten from the diet can lead to complete mucosal recovery, often after many months to years, but remission is incomplete in up to 30%–40% of patients despite a strict gluten-free diet (GFD).[12]

Need for Nondietary Therapy as an Adjuvant to the Gluten-Free Diet

There is currently no approved nondietary therapy for CeD; instead, management depends on life-long adherence to a strict GFD. Although this treatment is conceptually simple and pathophysiologically rational, it is difficult to achieve and maintain. In principle, each of the steps leading to tissue damage can be targeted to block disease progression (Figure 1).

A substantial proportion of patients with CeD (10%–40%) experience ongoing signs or symptoms of active CeD despite a GFD.[13,14] There is a variety of etiologies for "nonresponsive" CeD (NRCeD), but the most common is gluten ingestion, be it inadvertent or purposeful.[14] Other etiologies include irritable bowel syndrome, nongluten (wheat) allergic food sensitivities,[15,16] microscopic colitis, small intestinal bacterial overgrowth, and other food intolerances.[17]

Refractory celiac disease (RCeD) develops in 1%–2% of patients with CeD and is an important unmet medical need for CeD drug development. In type I RCeD (RCeDI), there is ongoing symptomatic enteropathy, usually with mild to moderate malabsorption, despite at least 12 months of treatment with a strict GFD. Patients with RCeDI manifest signs and symptoms similar to those of untreated or partly treated CeD. One theory regarding the etiology of RCeDI is that it reflects a heightened sensitivity to microcontamination of the diet, with small amounts of gluten.[18] Type II RCeD (RCeDII) is often associated with severe symptomatic enteropathy with malabsorption, despite at least 12 months of treatment with a strict GFD. RCeDII is characterized by the presence of aberrant mucosal T cells with malignant potential, incurring a high risk of development of enteropathy-associated T-cell lymphoma.[19]

Another important unmet medical need in CeD is the occurrence and risk of inadvertent gluten ingestion. This is especially likely to occur when patients consume food prepared by others—usually outside of their own home. Examples of "high-risk" exposure include eating in restaurants or cafeterias; attending social events, including family gatherings; and during travel.

Other challenges to those attempting to follow a GFD include their variable sensitivity to gluten exposures. Some develop severe symptoms (eg, diarrhea, abdominal pain, nausea and vomiting, and malaise) after ingesting tiny amounts of gluten.[18] In one study, the ingestion of just 50 mg of gluten daily (equating to approximately 1/40th the amount of gluten in a slice of normal bread) for 90 days led to a measurable deterioration in small intestinal histology.[20] A survey study on patients with CeD estimated a 70% likelihood of inadvertent or purposeful gluten ingestion.[21] This estimate agrees closely with a study that measured gluten contamination in food, urine, or feces using an enzyme immunoassay for gluten immunogenic peptides. Over a 10-day observation period, 67% of subjects showed gluten immunogenic peptide positivity in at least 1 sample.[22]

Gluten is highly pervasive in the Western diet and environment and can be found in unexpected places, such as medications or dietary supplements. Even more insidious is the contamination of naturally gluten-free foods, for example, oats, soy, or millet flour, with gluten.[23] Hence, following a strict GFD requires constant vigilance and careful planning, which is burdensome to many patients.[24] The rigors of avoiding gluten when eating away from home can exact a high social cost on patients and their families.[25] Furthermore, as gluten is so widely present in the food industry, a GFD may lead to low fiber intake or nutritional deficiencies.

One survey[26] found that a medication, or other intervention, that would allow those with CeD to safely and effectively withstand minor gluten contamination of the diet would be a welcome relief for >50% of patients with CeD and would improve quality of life substantially for patients with CeD (Table 1). It may be taken consistently or daily for those with ongoing symptoms or intermittently for those who mainly develop symptoms after dining away from their own home and kitchen. Moreover, it may alleviate symptoms in patients diagnosed with RCeDI, assuming that they have a very high sensitivity to microtraces of gluten. A more controversial application for such an agent would be to allow for a partial relaxation of the measures needed to maintain an exacting, constant, and absolutely strict GFD.

Tools to Test New Therapeutics

Preclinical studies can use in vitro, ex vivo, and in vivo animal studies. In vitro tools include systems to study the ability of therapeutics to mitigate the negative impact of gluten. Studies have used intestinal cell lines or epithelial organoids, assuming a relevant direct role of gluten in intestinal epithelial damage.[27] Preferably, systems that include immune cells, specifically gluten-reactive T-cell clones, have been used, either in isolation with HLA-DQ2 or -DQ8 expressing antigen-presenting cells or in mixed organoids that include lamina propria immune cells or IELs.[28] Ex vivo tools encompass the use of freshly isolated cells from small intestinal biopsies of patients with CeD or organ

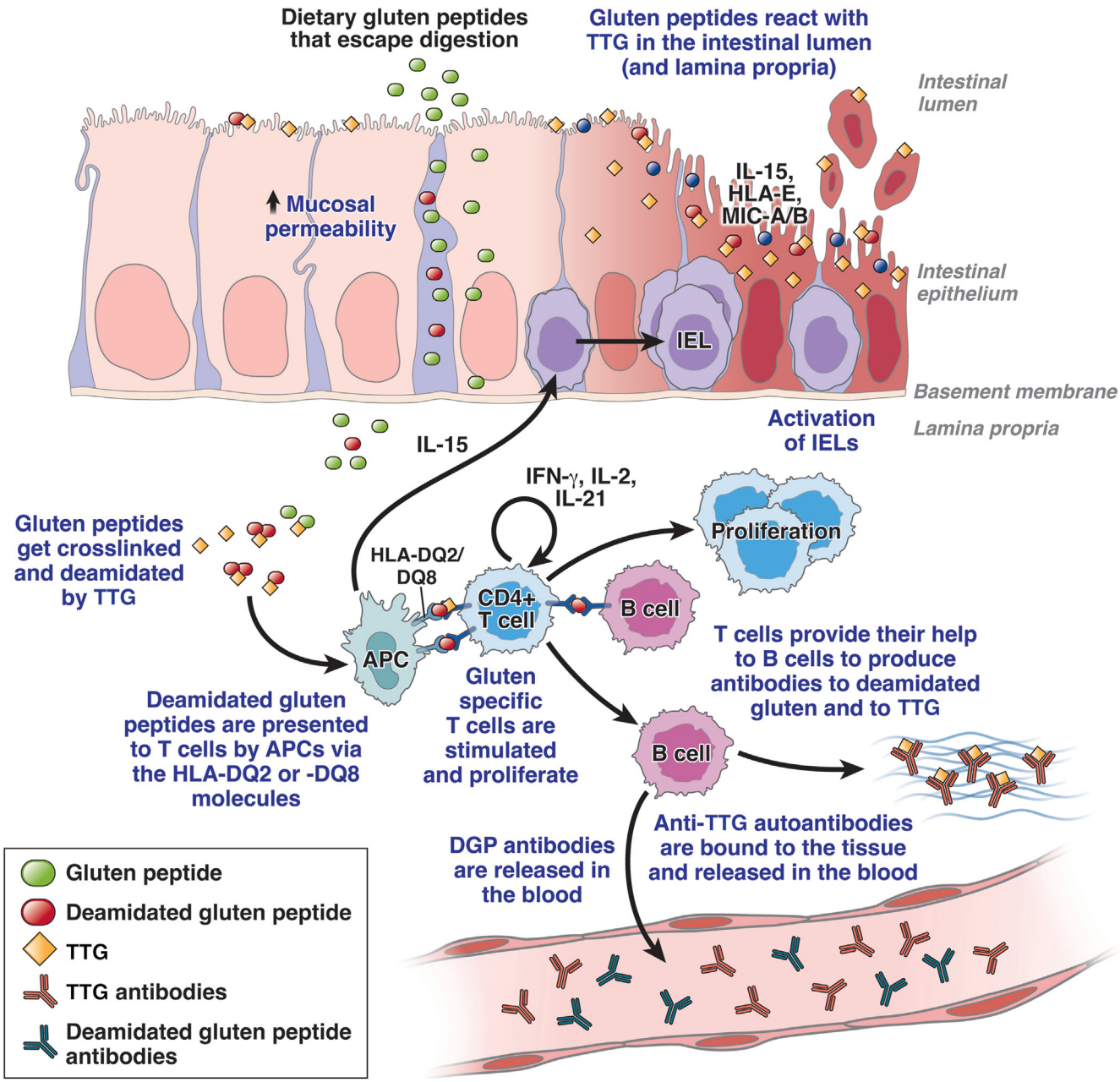

Figure 1. Pathogenesis of CeD. Undigested antigenic gluten peptides reach the lamina propria via "leaky" tight junctions (TJs), but also by means of active transepithelial transport. This uptake is enhanced by an increase in mucosal permeability (eg, by infections and medication, but also active CeD). There, and in the lumen, certain gluten peptides are deamidated by TTG, which is also increasingly released from various intestinal cells during inflammation. This deamidation induces a negative charge and improves their presentation by antigen-presenting cells (APCs) (especially macrophages, dendritic cells, and B cells) via the surface molecules HLA-DQ2 or -DQ8. This presentation expands gluten-specific inflammatory T cells that then release cytokines (eg, IFN gamma, tumor necrosis factor α), which in turn stimulate other cells, including (myo-) fibroblasts and macrophages to secrete and activate tissue-destructive enzymes (such as matrix metalloproteases). Those cytokines also contribute to the amplification of the adaptive anti-gluten immune response as well as to the activation of IELs. Gluten can also directly activate intestinal epithelial cells, and other wheat proteins like amylase-trypsin inhibitors that can stimulate myeloid cells via TLR4. Intestinal epithelial cells in active CeD show higher expression of stress molecules including MIC-A/B, HLA-E, and IL-15. Activation of intestinal epithelial cells along with pro-inflammatory cytokines produced in the lamina propria contribute to trigger a destructive cytotoxic T-cell response in the intestinal epithelium. The result is epithelial cell death and shedding, and inflammatory mucosal remodeling with villous atrophy and crypt hyperplasia. Furthermore, the activated T cells support the production of antibodies against gliadin, deamidated gliadin, and the autoantigen TTG, which form the basis of serologic CeD diagnostics. DGP, deamidated gliadin peptide.

Table 1. Unmet Medical Needs for Nondietary Therapy as an Adjuvant to the Gluten-Free Diet in Celiac Disease

Potential indication for therapy (approximate % of patients with CeD in need)	Comment	Applicable to children
NRCeD (10%–30%)	Persisting signs or symptoms of CeD despite patient's best attempts to follow a GFD. Other etiologies need to be considered/excluded, including irritable bowel syndrome, microscopic colitis, small intestinal bacterial overgrowth, and other food intolerances.	Less common in children
RCeDI[a] (1%–2%)	Although evidence is not definitive, RCeDI may represent a high sensitivity to dietary gluten microcontamination.	No
To protect against gluten exposures during unavoidable "high-risk" eating (>50%)	Examples include travel away from home, eating out in restaurants, or attending social functions.	Yes
To allow safe relaxation of the strict GFD to a very-low-gluten diet (all, if safe)	This is a controversial indication.	Controversial
Rescue medication (eg, enteric budesonide) to use immediately after inadvertent, substantial, gluten exposure (all)	The mechanism of action would need to be such that it facilitates rapid and effective interruption of the inflammatory cascade after gluten ingestion.	Controversial
To prevent chronic complications and/or autoimmunity (all, if safe)	It will be difficult to demonstrate efficacy for these indications within a time frame of only a few years.	Desirable

[a]Recent developments in RCeDII are described in the review on RCeDI and RCeDII by Malamut et al.[81]

cultures of duodenal biopsies.[10] In vivo mouse models that can yield an approximation to human pathology have been used. These include the transfer of gluten-specific T cells into immune-deficient mice[29,30] and human HLA-DQ2 and -DQ8 transgenic mice that, on gluten sensitization and oral challenge, can develop signs of mild CeD.[4,31–34]

Clinical studies are required for final proof of efficacy and safety of pharmacologic agents for patients with CeD. The study design may vary based on the mechanism of action of each compound and its intended use, such as gluten inactivation, induction of immunologic tolerance, protection of the intestinal barrier, prevention of gluten-induced immunogenicity, and reduction of gluten-induced symptoms. Most early-stage (phase 1b–2) clinical trials use a gluten challenge (eg, 3–16 g of gluten daily) in volunteers with CeD in clinical remission for 2–6 weeks along with the therapeutic compound vs placebo in a double-blind design. Baseline vs end of treatment centrally read histology (villus height to crypt depth ratio) is the primary end point, with several secondary end points, such as intraepithelial lymphocytosis, but also patient-related outcomes (PROs). Phase 2b–3 studies will then focus on final dose finding, long-term safety and efficacy, and end points that include both histology and PROs.[35] A combined clinical end point (ie, symptoms and histology) remains important because mere reliance on symptoms, even with improved scores, will also include patients with irritable bowel syndrome–related symptoms, including food sensitivities not due to CeD.

However, several studies suggest the feasibility of short-term proof-of-concept studies before this classical clinical study design. Anderson et al[36] established that subjects with CeD in remission challenged for 3 days with 2–3 slices of bread (ie, 8–9 g of gluten) per day had a temporary (days 3–6) significant increase of gluten-specific interferon (IFN)-gamma–positive T cells in the periphery, a very plausible surrogate of the CeD-specific T helper 1 T-cell response.[36] In analogy, patients with CeD show increased levels of HLA-DQ2 gliadin tetramers in the peripheral blood after a short-term gluten challenge, and tetramer positivity even permits diagnosing CeD in patients on a GFD.[37,38] An increase in circulating cytokines, especially IL-2, that peaks 4 hours after a single gluten challenge may serve as a valuable peripheral surrogate of gluten-induced intestinal inflammation.[39,40] Such a serum IL-2 peak has been found to associate with the onset of gastrointestinal symptoms, such as nausea and vomiting, elicited by oral gluten ingestion in adult patients with CeD on a GFD.[39] Moreover, an ex vivo test based on IL-2 release from freshly collected whole blood incubated with native gluten was found to correlate with peripheral IL-2 levels in response to the standard oral gluten challenge.[41] Using this assay and a refined, highly sensitive IL-2 assay, signals could even be detected without a prior gluten challenge. This supports the usefulness of the peripheral IL-2 response as a valuable surrogate of the gluten specific T-cell response without the need for a complex oral gluten challenge protocol. However, it remains to

be seen how far the extent of the initial IL-2 response predicts histologic damage or symptoms after long-term (low-dose) gluten exposure.

Regulatory Agencies in Celiac Disease Drug Development

The international regulatory pathways for drug development in CeD are in evolution—especially because there is, as yet, no single approved agent. Nonetheless, the US Food and Drug Administration has issued a draft guidance: "Celiac Disease: Developing Drugs for Adjunctive Treatment to a Gluten-Free Diet Guidance for Industry."[42] This guidance focuses on agents to improve both clinical symptoms and intestinal tissue injury (as proposed co-primary study end points) in patients with CeD who are already adhering to a GFD. Notably, the challenges are similar in the different regions and countries. Although this field is still in development, the published US guidelines suggest a combined histologic and PRO-based end point beyond phase 2a and 2 studies. The European Union countries, Australia, and New Zealand follow these guidelines, as also exemplified by the current largest phase 2b study (CEC-4; EudraCT Number: 2020-004612-97), which enrolled 400 patients with symptoms and (mild) histologic evidence of villous atrophy. Thus, study designs follow a consensus between the regulatory authorities and the stakeholders. Still, the relevant agencies will, on an individual basis for each development program, determine the scope, extent, and sites of the studies needed for regulatory approval.

Therapeutic Strategies

Strategy 1: Reduction of Gluten Antigenic Load

Genetically modified gluten and wheat. Several approaches have been explored to determine the feasibility of generating gluten and/or wheat that is safe for consumption by patients with CeD (Figure 2). For instance, introducing in gluten naturally occurring amino acid substitutions that are associated with a loss of T-cell stimulatory properties.[43] Because immunogenic epitopes occur mostly in gliadins, their genetic manipulation would not significantly change the desired baking quality of wheat, which largely depends on glutenin. E82 is a low-immunogenic variety of wheat, obtained using plasmid-mediated RNA interference to partly degrade messenger RNAs encoding major immunogenic gliadins, while sparing nonpathogenic glutenin.[44] Patients with CeD challenged with E82 bread showed lower gluten-induced IFN-gamma production of peripheral T cells and gluten immunogenic peptides in stools compared with those challenged with regular wheat bread, with no difference in gastrointestinal symptoms between the 2 groups in a 1-week challenge.[45]

Complete removal of several of the more than 100 gliadin genes or immunogenic epitopes in modern wheat has been achieved by gene editing with CRISPR/Cas (Clustered Regularly-Interspaced Short Palindromic Repeats/CRISPR associated protein), which reduced but did not eliminate gluten immunogenicity to a level that is safe for patients with CeD.[46–48] However, although the United States or Canada may be more open to permit gene-modified wheat, the European Commission concluded that a release of such gene-modified organisms into the environment, was "not fit for purpose."[49]

Sequestering agents. Another potential gluten-targeting therapy is the use of agents sequestering gluten in the intestinal lumen, preventing its translocation to the lamina propria (Figure 2). BL-7010 is a modified polystyrene selectively binding gluten in the gut lumen.[50] It attenuated tissue damage in gluten-sensitized HLA-DQ8 transgenic mice, as shown by an ameliorated villus height to crypt depth ratio and reduced intraepithelial lymphocytosis.[33]

AGY is an oral polyclonal egg yolk–derived anti-gliadin antibody that can bind gliadin and prevent its uptake in the gut.[51] Its safety has been shown in a phase 1 trial (ClinicalTrials.gov, Number: NCT01765647); results of a phase 2 trial (ClinicalTrials.gov, Number: NCT03707730) have not yet been disclosed.

Chitosan is a biocompatible aminopolysaccharide that imposes a different gluten reorganization producing in situ mechanically interlocked supramolecular assemblies between gluten and chitosan that lead in vitro to a decreased gluten digestibility, TTG deamidation susceptibility, and IFN-gamma production by intestinal cell lines generated from patients with CeD.[52]

Enzyme therapies. Another attractive therapeutic strategy aims at degrading dietary gluten peptides that are otherwise resistant to gastrointestinal proteases, thereby reducing the amount of immunogenic gluten peptides that can reach the lamina propria (Figure 2).[53]

Latiglutenase. Latiglutenase consists of 2 enzymes that, when combined, potently degrade gluten proteins. This enzyme cocktail has proved at least partial efficacy in several clinical trials.[54–56] In the most recent trial (ClinicalTrials.gov, Number: NCT03585478),[57] it was found that, compared with placebo, oral latiglutenase administration with a meal containing 2 g of gluten over a period of 6 weeks reduced severity of symptoms and associated mucosal damage. Moreover, analysis of urine samples taken during the trial demonstrated the efficient elimination of gluten fragments. As such, oral intake of latiglutenase offers an opportunity to prevent or diminish gluten-induced immune reactions leading to mucosal injury, provided that the enzyme cocktail is taken with gluten-containing foods.

Aspergillus niger-derived prolyl endoprotease. Aspergillus niger-derived prolyl endoprotease has been developed to promote luminal detoxification of gluten[58,59] and is currently in a clinical trial (ClinicalTrials.gov, Number: NCT04788797). This is a prospective, randomized, double-blind study in "real-life" patients with CeD to establish the effect of Aspergillus niger-derived prolyl endoprotease compared with placebo by measuring gluten immunogenic peptides in stool and urine, which is a surrogate of gluten exposure.

TAK062. Wolf et al[60] used in silico design to synthesize an optimized glutenase (TAK-062; Takeda). They demonstrated high efficacy to degrade gluten in vitro using a dynamic gastric model and showed its safety and tolerability in 14 healthy volunteers and 9 patients with CeD.

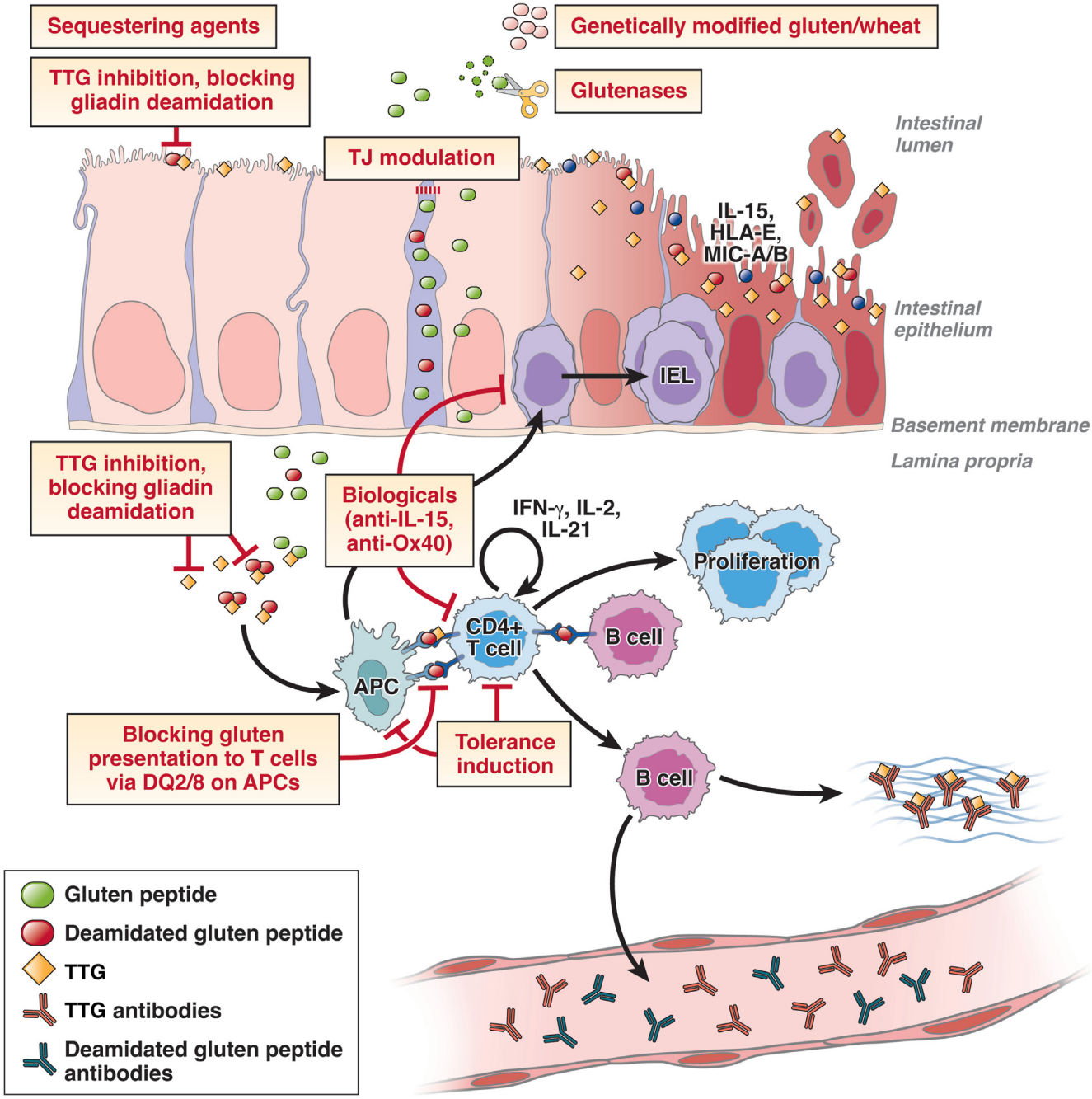

Figure 2. Approaches for pharmacologic therapies of CeD. Mechanism-based therapeutic approaches. For details refer to the text. APC, antigen-presenting cell; MIC, major histocompatibility complex class I polypeptide–related sequence; TJ, tight junction

Administration of TAK-062 (900 mg) to healthy volunteers before a complex meal containing 1–6 g of gluten resulted in 97%–99% degradation of 2 major immunogenic gluten peptides in the gastric fluid sampled 20–65 minutes after gluten ingestion. Although TAK-062 clearly appears to have superior gluten-degrading activity compared with previously reported glutenases,[53] the detection of >50 mg of residual gluten in the gastric fluid of several TAK-062–treated individuals may cause problems in some patients with CeD,[20] especially with everyday meals. A 50-mg daily gluten challenge for 3 months in patients with CeD in remission on a GFD caused a significant 20% mean decrease in villus length.[20] Although TAK-062 is a promising enzyme, further work is needed to assess clinical protection, possibly in combination with other therapies.

Tight junction modulation. Another reasonable therapeutic attempt is to reduce intestinal permeability that is a consequence of the small intestinal inflammation occurring in patients with CeD, and can contribute to the passage of more gluten peptides into the lamina propria (Figure 2).

Larazotide acetate (9 Meters Biopharma) is an octapeptide that is purported to prevent tight junction opening, thereby

reducing intestinal permeability and preventing lamina propria access by immunogenic gluten peptide fragments. However, the true mechanism of action of larazotide acetate is controversial.[61] Early-phase studies indicated that larazotide acetate was generally safe and well-tolerated.[62] When taken as an adjuvant to a GFD in a phase 2b study of patients with CeD with symptomatic CeD (NRCeD), it reduced symptoms compared with placebo.[63] A subsequent, placebo-controlled trial of larazotide acetate was the first ever phase 3 trial of a therapy for CeD, again based on PROs alone (ClinicalTrials.gov, Number: NCT03569007). The study was halted in June 2022 after an interim analysis indicated lack of significant benefit and that a clinically meaningful evaluation of efficacy for NRCeD would require enrollment of too many additional subjects.

IMU-856 (Immunic Therapeutics) is an oral, systemically acting, small molecule therapeutic agent that activates SIRT6 (Sirtuin 6), a transcriptional regulatory protein that supports intestinal barrier integrity and regeneration of the intestinal epithelium. It was reported to be safe and well-tolerated in a phase 1b clinical study,[64] where it also attenuated intestinal damage in volunteers with CeD in remission who were challenged with gluten. IMU-856 is now progressing to a phase 2 clinical study of safety and efficacy in NRCeD.

Strategy 2: Immune Modulation

Another set of therapies aims at blocking the immune response triggered by gluten in patients with CeD (Figure 2). One therapeutic strategy consists of blocking deamidation of gluten peptides by TTG, thus reducing their affinity for the HLA-DQ2/-DQ8 molecules on antigen-presenting cells and dampening the anti-gluten $CD4^+$ T-cell response in the lamina propria. Another approach would be to block the interaction of the HLA-DQ–gluten complex with the $CD4^+$ T cells in order to prevent their activation. Additional therapeutic options include molecules that can block inflammatory cytokines involved in CeD pathogenesis and their downstream signaling (ie, IL-15 and IL-21).[11]

Blocking deamidation of gluten peptides—tissue transglutaminase 2 inhibition—(ZED1227/TAK-227; Falk Pharma). The cellular enzyme TTG is both the CeD autoantigen and a central driver of the pathogenesis of CeD.[2–4,65] It deamidates certain dietary gluten peptides in the intestinal mucosa that escape gastrointestinal digestion, which facilitates their antigenic presentation by HLA-DQ2 or HLA-DQ8 and elicits activation and expansion of gluten-reactive $CD4^+$ T-cell clones.[6,7] Blocking the deamidation of gluten peptides may substantially reduce their binding and presentation to naïve T cells and the rise of a gluten-specific T-cell response. On this basis, Zhuang and Khosla[66] developed several small molecules working as TTG inhibitors that prevented gluten peptide deamidation. More recently, a highly specific and nontoxic TTG inhibitor (ZED1227), which lacked significant inhibition of 4 other major transglutaminases and showed no toxicity in preclinical and phase 1 clinical studies, was developed. This oral TTG-inhibitor ZED1227 prevented gluten-induced mucosal damage and improved PROs in a study of 160 patients with CeD in remission who were challenged with 3 g of gluten per day for 6 weeks.[67,68] Notably, in patients, ZED1227 appears to inhibit the enzyme both on the enterocyte surface and in the lamina propria.[69] Currently, ZED1227 is being tested in a phase 2b study of 400 patients with mild clinical as well as histologic NRCeD (EudraCT Number: 2020-004612-97).

Blocking gliadin presentation to T cells (DONQ52; Chugai Pharma). A key process in the inflammatory response in CeD is the specific recognition of gliadin peptides bound to HLA-DQ2.5/DQ8 by CD4 T cells. Blockade of this interaction would thus prevent the gliadin-specific inflammatory immune response (Figure 2). Recently, human T-cell receptor–like monoclonal antibodies have been developed through phage display technology that specifically interact with HLA-DQ2.5 loaded with one of the immunodominant gliadin peptides (DQ2.5-glia-α2). Detailed analysis of the interaction between this antibody and the HLA-DQ2.5–gliadin complex revealed a binding mode very similar to the gliadin-specific T-cell receptors commonly found on CD4 T cells of patients.[70] Consequently, this antibody specifically inhibited activation of such CD4 T cells by HLA-DQ2.5–positive antigen-presenting cells loaded with the DQ2.5-glia-α2 epitope, both in vitro and in a humanized mouse model. Such an antibody-based approach is currently being tested in a phase I clinical trial (ClinicalTrials.gov, Number: NCT05425446). Although promising, it should be noted that this approach may not eliminate the disease-causative memory T cells completely, and may require repeated application of the antibody.

Blocking cytokine signaling and immune modulation. Cytokines produced by gluten-activated $CD4^+$ T cells (IFN gamma, IL-2, and IL-21) and by stressed myeloid and epithelial cells (IL-15) orchestrate further T-cell expansion and activation of CT-IELs, promoting villous atrophy (Figure 1).[4,10,71]

Anti–IL-15. Based on these insights, anti–IL-15 antibodies to inhibit epithelial damage[72] or to oppose the outgrowth of malignant IELs in RCeDII[73] have been tested in the clinic (Figure 2). An IL-15 blocking monoclonal antibody (PRV-015; ClinicalTrials.gov, Number: NCT04424927) was used in phase 2 studies of patients with CeD with gluten provocation, with NRCeD and RCeDII, with varying degrees of success.[72,73] A phase 1b trial with an antibody with higher affinity was successfully completed in late summer 2023 (Caly-002; ClinicalTrials.gov, Number: NCT04593251). TEV-53408 is an IL-15 blocker currently recruiting in a phase 1b trial.

T-cell intestinal recruitment. Another potential therapeutic intervention involves the inhibition of immune cell trafficking. Vedolizumab, a monoclonal antibody anti-$\alpha4\beta7$, an integrin involved in T and B cells homing to the gut,[74] has already been approved for use in ulcerative colitis and is now being evaluated in patients with CeD (ClinicalTrials.gov, Number: NCT02929316). Another small molecule, CCX282-B, orally bioavailable, selective, and potent antagonist of human CCR9-mediated Ca^{2+} mobilization and chemotaxis, has been implemented in Crohn's disease[75] and also tested in a double-blinded, placebo-controlled, randomized, phase 2 trial in CeD (ClinicalTrials.gov, Number: NCT00540657), but the results have not been disclosed, likely due to lack of efficacy.

Anti-Ox40L (amlitelimab; Sanofi). OX40L is the ligand for OX40 (TNFRSF4, a member of the tumor necrosis factor receptor family). It is expressed on antigen-presenting cells, such as type 2 dendritic cells, macrophages, and B cells, whereas OX40 is expressed by activated $CD4^+$ T cell, natural killer, natural killer T cells, and neutrophils. Importantly, ligation of OX40 by interaction with OX40L promotes the development of memory T cells.[76,77] Up-regulation of the OX40/OX40L pathway is found in multiple autoimmune diseases. In a study of patients with active CeD, 25% of lamina propria $CD4^+$ T cells were positive for OX40 by flow cytometry.[78] Moreover, in mice, $OX40^+$ IELs display greater cytotoxicity compared with OX40- IELs.[79] A recent study demonstrated Ox40 circulating and intestinal $CD4^+$ T cells in patients with active CeD vs healthy controls, with highest OX40 expression in gluten tetramer–positive $CD4^+$ T cells.[80] Amlitelimab is a human anti-OX40L IgG4 subclass monoclonal antibody that targets human OX40L to block interaction with its receptor OX40 (Figure 2). Patients with NRCeD will be randomized into groups receiving subcutaneous amlitelimab or placebo every 4 weeks for 12 weeks.

Enteric-release budesonide. Enteric-release (ER) budesonide has been used (off label) to treat NRCeD and RCeD (see the review on RCeDI and RCeDII by Malamut et al[81]). Budesonide is a corticosteroid with a high first-pass hepatic metabolism. ER budesonide can reduce small intestinal inflammation in CeD. Reports of efficacy are anecdotal however, as no controlled trials have been performed. One study of ER budesonide 9 mg daily in 42 patients with NRCeD reported that 57% showed a clinical response with overall significant reductions in diarrhea and fatigue. Mucosal recovery was observed in 11 of 24 patients with follow-up duodenal biopsies. Clinical recurrence was frequent after tapering ER budesonide.[82–84] In some studies, open-capsule ER budesonide was used.[82] ER budesonide appears to be less effective in (mild) RCeDII compared with RCeDI. ER budesonide has also been used as a rescue therapy for patients with CeD who have recently experienced an inadvertent acute gluten exposure.[85] It has also been given to patients, generally children, presenting with the rare condition of celiac crisis, a potentially life-threatening complication of CeD leading to severe diarrhea and a protein-losing enteropathy, generally treated with systemic corticosteroids along with GFD.[86]

Strategy 3: Tolerance Induction

The "holy grail" in immune-mediated diseases is the induction of immune tolerance. The fact that the formation of immunologic memory is a key feature of the adaptive immune system complicates effective and long-lasting tolerance induction. CeD is no exception in this complex scenario. The identification of the repertoire of immunodominant gluten peptides recognized by gluten-specific T cells[87] has significantly advanced the implementation of this approach. Below, we summarize a number of recent approaches to induce tolerance to gluten in patients with CeD (Figure 2).

NexVax2 (ImmusanT). Based on the finding that all patients with CeD responded to a limited set of 3–4 key immunodominant gliadin-derived peptides, ImmusanT aimed to introduce tolerance to those epitopes. Patients received either placebo or synthetic gliadin peptides that incorporated the deamidation pattern naturally introduced by the activity of TTG subcutaneously, followed by a subsequent oral gluten challenge and monitoring of disease activity. Although initial studies showed promising results, a phase 2 clinical trial was terminated after an interim analysis indicated that the approach failed to protect from subsequent gluten exposure.[39]

Tak-101 (Takeda). A different immunotherapy examined the use of intravenous infusions of negatively charged biodegradable 500 nm poly-DL-lactide-co-glycolide nanoparticles loaded with a mixture of native and deamidated gliadin peptides (TAK-101).[88] Prior mouse models of autoimmunity and delayed hypersensitivity demonstrated that such nanoparticles, loaded with the respective autoantigens or allergens, mainly addressed antigen-presenting cells in the spleen, reducing activation or inducing anergy in specific effector $CD4^+$ and $CD8^+$ T cells, while stimulating expansion of antigen-specific regulatory T cells.[89] TAK-101 could also reduce the activation of gluten-specific $CD4^+$ T cells in the preclinical CeD mouse model generated by transfer of gluten-specific T cells.[30] After a phase 1 safety trial, 34 patients with CeD in remission received 2 infusions of either TAK-101 or placebo on days 1 and 8. Oral gluten challenge was started at day 15 with 12 g/d for 3 days and then 6 g/d until day 29. Strikingly, the number of circulating gliadin-sensitized IFN-gamma–producing T cells increased between day 1 and day 29 in the placebo but not in the TAK-101 group, suggesting successful desensitization and achieving the primary study end point. Coincidently, circulating $CD4^+$ T cells with signatures of immune activation like CD38 increased in the placebo-treated subjects, but not in the TAK-101–treated subjects.[88] Overall, this phase 2a proof-of-concept study's results are encouraging; yet, final conclusions as to robust efficacy or duration of effect cannot be drawn.

KAN-101 (Anokion-Kanyos). KAN-101 is a compound composed of a liver-targeting glycosylation signature conjugated to a deamidated peptide from α-gliadin, which binds HLA-DQ2.5 and is designed to induce tolerance to gliadin by promoting tolerogenic pathways in the liver and preventing T-cell activation in response to gluten exposure in patients with CeD.[90,91] After preclinical studies in animals showed a good safety profile in vivo, the first-in-human, double-blind, controlled, phase 1 trial was carried out in 41 patients with CeD carrying the HLA-DQ2.5 genotype and on a GFD. This showed an acceptable safety profile and a rapid systemic clearance (6 hours) of KAN-101 with no accumulation after repeated dosing. The most common adverse events were gastrointestinal symptoms, such as nausea, diarrhea, abdominal pain, and vomiting, consistent with symptoms reported by patients with CeD on a GFD after gluten ingestion.[92] The drug is currently undergoing a phase1b/2a clinical trial (ClinicalTrials.gov, Number: NCT06001177), which should be completed in 2025.

TPM502 (TOPAS). TPM502 is a mixture of polymer-coated iron oxide nanoparticles carrying antigenic peptides comprising major deamidated gluten epitopes for HLA-DQ2.5. Those nanoparticles are designed to deliver antigens to sinusoidal endothelial cells in the liver, which have shown immune-tolerogenic capabilities in a mouse model of multiple sclerosis.[93] It is currently under investigation in a multicenter, double-blind, randomized, placebo-controlled, phase 2a clinical trial (ClinicalTrials.gov, Number: NCT05660109) that aims to test its safety, tolerability, and pharmacodynamic profiles in adult patients with CeD, along with immune tolerance and symptom severity after gluten challenge.

Challenges for Pharmacologic Treatment in Children

There are currently no ongoing drug developmental clinical trials in children with CeD. The scenario of pediatric CeD is indeed different from that of adults. Although extrapolation of data obtained in adults will improve efficiency and success of pediatric drug development, it implies similar disease course, clinical presentation, diagnostic algorithms, and expected response to therapy. However, several parameters, such as dosing and safety cannot be fully extrapolated (Table 2). One example is also the amount of gluten needed for challenges in children to elicit tissue damage and clinical symptoms that serve as end points in phase 2 clinical trials. Most gluten challenge studies have indeed been performed in adult patients, and little is known about clinical, histologic, and immunologic end points in children.

Although CeD pathogenesis does not differ substantially in children, clinical presentation varies depending on age, such as stunted growth, appetite loss, or the classical gastrointestinal symptoms. Children are often less symptomatic in response to voluntary or inadvertent gluten intake, which will require different pediatric PROs in response to gluten challenge compared with adults. Moreover, depending on age, PROs may not be scored by the patients, but by their caregivers, making the assessment even more complex, as age-dependent validated tools are not available.

According to the latest European Society for Paediatric Gastroenterology Hepatology and Nutrition guidelines,[94] the diagnosis of CeD in children may be made independent of the presence of symptoms. Moreover, when anti-TTG titers are at least 10-fold above normal, evaluation by endoscopy and small intestinal biopsy may be omitted, shifting the center of the diagnostic algorithm from histopathology to anti-TTG autoantibodies. Notably, autoantibody titers show only little correlation with histologic damage, especially in children.[95] On this basis, serology is not currently included among the primary outcomes of adult clinical trials, while histology and symptoms are.

Children with CeD respond to a strict GFD more quickly and more fully than adults. The rate of complete mucosal healing in children reaches up to approximately 80%,[96] and a follow-up biopsy is not generally performed unless children present with new or recurring symptoms, have persistently elevated serology or additional upper gastrointestinal comorbidities. The implementation of the non-biopsy diagnostic approach, along with higher healing rates than in adults, may make the idea of 2 upper endoscopies, 1 at baseline and 1 to assess mucosal healing in response to the administration of a drug, difficult to accept for families of children who did not even undergo endoscopy at diagnosis. That histologic end points can be omitted in pediatric phase 2–3 trials is unlikely, as there is currently no better proxy for mucosal healing and how families will receive this scenario requires further exploration.

Although gluten detoxification strategies intended to reduce the risk of gluten contamination when eating out may also be a valid option in pediatrics, there is no evidence of RCeD in children and few cases of NRCeD.[97] Thus, some immunomodulatory options may be less relevant in children. In contrast, promoting tolerance to gluten may be more promising in children than anti-inflammatory agents that may be accompanied by long-term and less predictable adverse effects, due to the early-life immune intervention and longer life expectancy and expected treatment duration. Indeed, drugs intended for chronic administration should

Table 2. Differences Between Children and Adults in Drug Development Clinical Trial

Variable	Adults	Children
Diagnosis	Histology	Serology ± histology
Mucosal healing assessment by histology	Slow Frequent	Faster than adults Infrequent
PRO	Validated	Age dependent/not validated
Gluten challenge protocols	Standardized	Not standardized
Safety/risks	Assessed in phase 1/2	Not accurate extrapolation from adults (longer life expectancy)
Immunologic outcomes	IL-2 release IFN gamma Enzyme-linked immunospot	Not standardized

Table 3. Therapeutics in the Pipeline and Their Applicability in Adults and Children

Therapeutic strategy	Adults	Children
Reduction of antigenic load		
Genetically modified gluten and wheat	Applicable	Applicable
Sequestering agents	Applicable	Applicable
Enzyme therapies	Applicable	Applicable
Tight junction modulation	Applicable	Applicable
Immune modulation		
Blocking deamidation (anti-TTG)	Applicable	Applicable
Blocking gliadin presentation to T cells (DONQ52)	Applicable	Applicable
Blocking cytokine signaling (anti–IL-15, anti–IL-15/IL-21)	Applicable	Controversial
Blocking intestinal T-cell recruitment	Applicable	Controversial
Enteric release budesonide	Applicable	As needed
Tolerance induction		
Nanoparticles, TAK101-TPM502	Applicable	Applicable
KAN-101	Applicable	Applicable

have an excellent long-term safety profile and be available at a reasonable cost, especially because a GFD is a safe, effective, and available option (Table 3).

In the pediatric population the prospect of preventive, along with therapeutic, options becomes very relevant, in particular for specific patient populations like children at high genetic risk (first-degree relatives) and potential patients with CeD. The latter group includes patients displaying positive serology in the absence of tissue damage who are at risk of developing full-blown disease over time.[98] Those patients may benefit from strategies that may delay or even prevent disease onset or slow its progression, similar to type 1 diabetes.[99]

However, strategies to prevent CeD (eg, antiviral vaccines and early feeding strategies), may represent a primary preventive strategy for children at high-genetic risk, like HLA-DQ2.5 homozygous girls, who have a 30% risk of developing CeD over time.[100] Future studies will further explore those options in distinct pediatric populations.

Potential Clinical Indications for Nondietary Therapy to Complement a Gluten-Free Diet

As described in the overview and in Table 1, there are multiple (potential) indications for pharmacologic therapy in CeD. When a safe and effective pharmacologic therapy for CeD receives regulatory approval, it is likely to be eligible for insurance reimbursement, given a demonstrable medical need and the lack of available nondietary therapies for specific indications (Table 1). At present, the indication of NRCeD is the primary focus of clinical trials. Novel therapies will aim to reduce symptoms and to improve histologic abnormalities in NRCeD. Such agents will be welcomed by patients with NRCeD and are likely to be used by both patients and their physicians.[101] Similarly, an approved agent will be welcomed and used by the smaller number of patients with RCeDI and RCeDII.

Concluding Remarks: From Treating to Curing Celiac Disease

A strict and lifelong GFD is currently the safest and only approved therapy for CeD. However, new therapeutic strategies are no longer just a dream, and several phase 1b–3 drug trials are under way. While summarizing the current knowledge on novel therapeutic avenues for patients with CeD, this review highlights the importance of the availability of different potential therapeutic options that can be considered for different patient groups and settings.

A desirable option will be primary prevention for children at high genetic risk, and secondary prevention for potential patients with CeD. Moreover, as many patients are not symptom-free despite a GFD (NRCeD), as a strict GFD is often difficult to maintain, leading to a reduced quality of life, therapies adjuvant to a GFD are needed and desired by many patients. This may also include a standby medication protecting from inadvertent gluten exposure when eating out or traveling. Finally, re-establishing immune tolerance to gluten might be a cure for CeD, albeit the presence of immune memory cells may render long-lasting tolerance hard to achieve. Currently, there are no therapeutic approaches able to contrast both circulating pathogenic central memory and tissue-resident effector memory T cells. Moreover, the persistence of tolerance restoration promoted by therapeutics currently under investigation in CeD needs to be addressed. A durable restoration of tolerance will require deletion or reprogramming of autoreactive T cells, possibly in combination with induction of antigen-specific regulatory T cells. This will likely be achieved only through a combination of therapeutic approaches.[102] Furthermore, an intestinal tolerogenic immune environment, as possibly achieved by a tolerizing therapy for CeD, may be jeopardized by intestinal infections that promote innate immune responses and are often combined with barrier defects, leading to an aggressive immune environment.[103,104]

Currently, most adjuvant therapies are under development for patients with persistent symptoms, positive

serology, or tissue damage, despite a strict GFD, including NRCeD and RCeDI, but these therapies may benefit a broader patient population later.

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Conflicts of interest
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