Current and emerging therapies for coeliac disease

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Key points

-At present, a gluten-free diet (GFD) is the only effective treatment for coeliac disease, but it is associated with several possible challenges including economical and societal high burden, inferior quality of life and sometimes inadequate response.

-Increased understanding of the pathogenetic process in coeliac disease has revealed various therapeutic targets for future drugs that could complement or replace the GFD.

-Novel therapeutic strategies include approaches to detoxify gluten already in the gastrointestinal tract by sequestrants or peptidases.

-Other investigational approaches comprise blocking intestinal epithelial permeability or the enzymatic activity of transglutaminase 2.

-Restoring immune tolerance to gluten or targeting the gluten-induced immune activation has also been investigated as possible therapeutic options.

-The most advanced drug candidates have now entered phase III clinical trials.

Abstract

Coeliac disease is a common enteropathy that occurs in genetically susceptible individuals in response to ingestion of gluten proteins present in wheat, rye and barley. Currently, the only available treatment for the condition is a strict, life-long gluten-free diet that, despite being safe and often effective, is associated with several challenges. Due to the high cost, particularly restrictive nature and perception of decreased quality of life associated with the diet, some patients are continuously exposed to gluten, which prevents an adequate disease control. Moreover, a subgroup of patients does not respond to the diet adequately and healing of the small bowel mucosa can be incomplete. Thus, there is a need for alternative treatment forms. The increasingly understood pathogenetic process of coeliac disease has enabled the identification of various targets for future therapies. Multiple investigational therapies ranging from tolerogenic to immunological approaches are in the pipeline and several drug candidates have entered phase II/III clinical trials. This Review gives a broad overview of the different investigative treatment modalities for coeliac disease and summarizes the latest advances in this field.

Introduction

Coeliac disease is one of the most common food-related chronic conditions with a worldwide prevalence of 1.4% and is therefore a considerable global public health concern[1]. Patients with coeliac disease can have various types of gastrointestinal and extraintestinal symptoms such diarrhea, abdominal pain, anemia and osteoporosis that differ in severity but the patients may also be apparently asymptomatic[2]. The disease pathogenesis is driven by the ingestion of dietary gluten present in wheat, rye and barley resulting in small-bowel mucosal villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis by mechanisms that are increasingly understood[3]. This characteristic small-bowel mucosal damage has been the cornerstone of the diagnosis in most countries, although serological methods including the detection of tranglutaminase-2 (TG2) autoantibodies are commonly used in the diagnostic workup[4,5].

Currently, the only accepted treatment for coeliac disease is a strict, life-long gluten-free diet (GFD), which usually results in alleviation of the symptoms and improvement of the small-bowel mucosal damage[6]. Adherence rates to GFD are generally above 80–90%[7], but due to the wide use of wheat in food, gluten can be difficult to avoid, resulting in inadvertent gluten exposure[8]. Furthermore, the diet is socially and economically burdensome with possible negative effects on quality of life[9,10]. In addition, despite the diet, up to 30% patients have persistent symptoms[6] and the healing of the small-bowel villous atrophy is reported to be suboptimal even in 60% of patients so the enteropathy can persist [11,12]. These issues raise the need for new non-dietary therapeutic approaches for coeliac disease. Several therapeutic targets have been identified and a number of clinical trials have either been completed or are currently underway. This Review will give a broad overview of the latest advances in the

development of novel therapeutic approaches in coeliac disease, ranging from a vaccine to enzymatic cleavage of gluten to immunological approaches.

Pathogenesis

Due to the high proline content of gluten (BOX 1), gastrointestinal digestive enzymes are not able to degrade gluten proteins efficiently[13]. Thus, long oligopeptides, which are able to induce adaptive and innate immune response in patients with coeliac disease, persist in the intestinal lumen (FIGURE 1). The peptides serve as substrates for the endogenous enzyme TG2, the coeliac disease-associated autoantigen, which catalyzes their deamidation converting specific glutamine residues to glutamic acid[14]. The prevailing hypothesis has been that the TG2-mediated deamidation occurs in the lamina propria, to which the peptides would gain access due to increased intestinal epithelial permeability[15]. However, gluten peptides can also be detected in the urine of healthy individuals, indicating that they can traverse through the intestinal epithelium and lamina propria in normal physiological conditions[16], thus challenging the concept of increased intestinal epithelial permeability in coeliac disease specifically. In this light, the proposition that the deamidation might occur in the intestinal lumen by virtue of catalytically active TG2 released from shed enterocytes is interesting[17]. Regardless of the site, deamidation increases the gluten peptide binding affinity to coeliac disease-predisposing human leukocyte antigen (HLA)-DQ2 or DQ8 heterodimers expressed on antigen-presenting cells[18]. This process provides an explanation for why these two particular HLA-haplotypes confer the highest genetic susceptibility for coeliac disease[19]. The HLA-bound peptides are presented to gluten specific CD4+ T cells, which leads to their activation, clonal expansion and secretion of proinflammatory cytokines including IFNγ and IL -21[3]. Although a broad range of gluten peptides can stimulate pathogenic T cells in patients with coeliac disease, a limited number of immunodominant epitopes can elicit a

response in most patients. In wheat, the most immunogenic gluten epitopes reside in α-gliadin and ω-gliadin[20]. However, it is likely that additional yet unreported epitopes do exist[20]. Interestingly, peptides from barley (hordein) and rye (secalin) can trigger populations of pathogenic T cells discrete from those triggered by wheat gluten, an important consideration in the design of new coeliac disease therapies[21,22]. In addition to contributing to the generation of an inflammatory milieu permissive for tissue destruction, gluten-specific T cells also promote a cascade of events leading to the production of TG2-autoantibodies and antibodies targeting deamidated gluten peptides[23].

The small-bowel mucosal lesion in coeliac disease is characterized by an increase of intraepithelial lymphocytes (IELs), crypt hyperplasia and villous shortening in the duodenum and it is thought that a complex interplay between the adaptive and innate immunity is needed for the development of such enteropathy (FIGURE 1). The characteristics of the innate immune response in coeliac disease are not completely understood but according to current concept are mediated by key cytokines, IL-15 being the most studied. The chronic upregulation of IL-15 in the epithelium and lamina propria is a hallmark of coeliac disease and it positively correlates with the degree of mucosal damage in patients[24]. In a mouse model of coeliac disease, a parallel overexpression of IL-15 was required for villus atrophy through activation of intraepithelial cytotoxic lymphocytes, leading to intestinal epithelial cell destruction[25]. The same study also demonstrated a role for IL-15 in the induction of T helper (Th)1 responses in the lamina propria, thus exemplifying co-operation between the adaptive and innate immunity.

Innate responses in coeliac disease could be triggered by multiple factors, including different microbial agents[26,27] (FIGURE 1). National and multicentre follow-up studies published in

the past few years indicate that gastrointestinal infections generally or with a specific microbe such as enterovirus have a role in increasing the risk of coeliac disease in children with genetic susceptibility[28-30]. In agreement, experimental studies support the hypothesis that microorganisms might play a part in the development of food sensitivities in coeliac disease through several mechanistic pathways[31]. For instance, in an animal model, viral infections have the capacity to trigger characteristic inflammatory pathways at sites where responses to dietary antigens take place, blocking regulatory T cells and inducing adverse responses to gluten instead[26].

The gold standard: gluten-free diet

Gluten is a complex mix of many related proteins with similar amino acid sequences (BOX 1). In wheat, gluten consists of gliadin and glutenin and makes up approximately 70% of the protein content and is, therefore, a major structural component of the cereal[32]. Wheat gluten is encoded by around hundred genes and for this reason and, along with generally negative attitude towards genetically modified foods, efforts to genetically engineer non-immunogenic wheat while maintaining important properties of the grains has proven challenging[33].

Strict and permanent exclusion of all gluten-containing products from the diet remains the only recommended treatment for coeliac disease and usually results in improvement or resolution of enteropathy and symptoms[6]. However, data suggest that not all patients respond to the diet adequately, either symptomatically or histologically, and the primary reason for this aspect is inadvertent gluten exposure[11,34]. It is estimated that in 0.3–0.4% of patients with coeliac disease, despite a strict GFD and exclusion of alternative causes for the enteropathy, the malabsorption and villous atrophy persist[34]. This condition, termed refractory coeliac disease (RCD) currently has no approved therapies. RCD is divided into

two types, RCDI and RCDII, the latter identified by the presence of aberrant clonal T cells in the intestinal epithelium, a high risk for development of enteropathy-associated T cell lymphoma and a poor prognosis[34]. RCDI generally responds well to therapy with corticosteroids (such as budesonide) and immunosuppressive medications such as infliximab and thiopurines, but patients with RCDII do not[34] . A key goal of RCDII therapy is to destroy the premalignant clonal T cell population, which is why chemotherapy (for instance with cladribine, a purine analogue), and autologous haematopoietic stem cell transplantation have been utilized with mixed success[34]. Besides the problems associated with unresponsiveness to GFD, the diet itself can be both complex and burdensome. Challenges with the diet can be grouped into three major categories: ensuring adequate gluten avoidance; maintaining a well-balanced diet; and minimizing burden and social restrictions for patients (BOX 2).

Studies measuring gluten amounts in food, urine and stool have revealed that patients on a GFD are at risk of inadvertent gluten ingestion due to the ubiquity of wheat in our food supply[35,36]. Although the ingested quantities are likely small, the clinical consequences of these are unknown. The sensitivity to gluten varies widely between individuals, probably due to a combination of genetic, immune and microbial factors[27,37,38]. Some patients exhibit minimal symptoms and intestinal injury even with prolonged gluten challenge and others develop severe symptoms and enteropathy even with minimal exposures[39,40]. For some individuals with coeliac disease, as little as 50 mg of gluten can activate disease[39], which explains why cross-contamination of food by gluten is problematic and how stringent ‘gluten contamination elimination diets’ excluding practically all processed foods even those labelled gluten-free can be helpful.

Gluten-containing grains are the foundation of the diet in many parts of the world (BOX 1). For this reason, removal of gluten from the diet can theoretically result in much more dramatic nutritional shifts than avoidance of other foods, for instance a single foodstuff in allergy or dairy in lactose intolerance. If the overall diet is unbalanced, patients with coeliac disease might be at risk of insufficient consumption of fibre, iron, folate and zinc[41,42], and also excess intake of heavy metals (including arsenic[43]) and weight gain[44].

Food is a central feature of human society and sharing meals is a commonality that transcends virtually all cultures. In addition, wheat is a basic foodstuff in most populations and mitigation of inadvertent gluten exposure can require constant vigilance. Gluten-free products are also often more expensive than their gluten-containing counterparts[45]. For these reasons, maintaining a GFD can be burdensome to patients and caregivers, leading in some cases to anxiety, precipitation or exacerbation of disordered eating, social isolation and impaired overall quality of life[10,46–48]. These issues could be alleviated by adjunctive therapies discussed herein.

Insights into treatment trials

In general, therapeutic development in coeliac disease follows the traditional phase I–IV progression of the Food and Drug Administration (FDA) categories with a few exceptions. A drug that targets gluten or a process found in the general population such as cytokine activity might be initially assessed in healthy volunteers as usually in phase I studies. Conversely, for a drug targeting loss of tolerance to gluten, studies in healthy volunteers might be uninformative, and phase I studies in patients with coeliac disease should be considered. In addition, therapeutic development for RCD, would be more similar to that used for other rare diseases, including some cancers. In these cases, phase I studies lead directly into small,

potentially uncontrolled, phase II/III studies for approval, followed by confirmation of therapeutic benefit in post-marketing studies.

Early stage trials of therapies with unproven mechanisms of action typically involve reintroduction of gluten to the diet e.g. a gluten challenge to patients with coeliac disease on GFD. This step enables efficient testing of a drug's ability to prevent or reduce immune activation due to gluten exposure. However, this process could be of limited relevance for therapies intended for RCD. The amount of gluten used has varied between the studies from 0.5 g at once[49] up to 16 g daily[50] complicating the comparison of the efficacy of the drug candidates thus raising a need for standardization of the challenge. The difficulty is the great individual variance in the gluten levels (0.05–5 g/day or even higher) and time of exposure (1–12 months or even longer) that are needed to cause abnormalities in small-bowel mucosa or provoke symptoms [39,40,51]. Furthermore, symptoms such as abdominal pain and diarrhea provoked by gluten challenge are not specific for coeliac disease and could be triggered by other dietary components such as fermentable carbohydrates[52].

Once a therapy is proven to be effective during gluten challenge, later studies should reflect the target indication, in most cases assessing change in symptoms and small intestinal mucosal damage in patients who are not adequately controlled with a GFD only. However, there is no consensus on the optimal combination of measurements of disease activity in clinical trials[53] (TABLE 1). Patient-reported outcomes can reflect the practical everyday life benefits of a drug, but their subjectivity, placebo and/or nocebo effects and natural fluctuation especially in non-specific symptoms limit their use. As histology is the only outcome associated with prognosis[12], it has been suggested to be combined with clinical outcomes such as symptoms alone or together with serology measuring for instance TG2 autoantibodies

to ensure that the reported changes are reflective of change in disease activity. It is notable that clinical trials could associate with a Hawthorne effect (e.g. possible effects of the awareness of being studied to study patients' behavior)[54] both in symptoms and histology, likely due to a combination of placebo effect and true reduction in gluten exposure. To prove a therapy safe and effective in the real world, use of low-dose gluten exposure to mitigate observer effects in later stage trials could be considered.

Novel therapeutic approaches

A rational approach to developing new therapies for coeliac disease is made possible by our knowledge of its pathogenesis, and several drug candidates targeting different steps in the pathogenesis have been introduced (FIGURE 1). The investigated approaches are described herein, starting from the frontrunners that have already entered phase III or II clinical trials.

Blocking intestinal epithelial permeability

The increased intestinal permeability implicated in coeliac disease is suggested to be contributed by both transcellular and paracellular epithelial permeability, and apical junctional protein complexes called tight junctions are key components in the latter process[15]. Currently, the only drug in phase III, larazotide acetate (also known as AT-1001 and INN-202), is a synthetic, locally acting 8-amino acid peptide that has been shown to decrease intestinal permeability by enhancing tight junction assembly and actin rearrangement *in vivo* and *in vitro*[55]. Its safety and effects have been evaluated in both phase I and phase II trials (TABLE 2). Orally administered larazotide was evaluated in a phase I randomized, double-blind trial in which 14 patients with coeliac disease received larazotide 12 mg and 7 patients placebo for 3 days[56]. The proportion of patients experiencing overall gastrointestinal symptoms and diarrhoea during the 1-day gluten challenge of 2.5 g was lower among those

who received larazotide compared to the placebo group. However, the change in intestinal permeability measured with urinary lactulose-to-mannitol (LAMA) ratio did not differ significantly between the larazotide and placebo arms. There were no serious adverse effects.

In the following phase IIa study with altogether 86 coeliac disease patients on GFD, LAMA ratios varied highly in outpatients and did not differ significantly between those on a GFD and those undergoing gluten challenge of 2.4 g daily or between larazotide and placebo. In addition, lower doses of larazotide limited the worsening of the severity of gluten-induced gastrointestinal symptoms [57]. In a double-blind multicenter phase IIb study with 184 patients with coeliac disease, the results were similar, as LAMA ratios did not differ between larazotide and placebo and gluten-induced (2.7 g daily) symptoms were prevented, although only with 1 mg of larazotide three times per day but not with the higher doses[58]. In this trial, all larazotide doses reduced statistically significantly the increase in TG2 antibody levels during gluten challenge compared with placebo. When larazotide was studied in a multicentre, randomized, double-blind trial in 342 patients with coeliac disease who had persistent symptoms despite a strict GFD, the lowest larazotide dose of 0.5 mg administered three times per day was shown to significantly reduce gastrointestinal symptoms during a 12-week study period, whereas the doses of 1 mg or 2 mg did not differ from placebo[59]. A phase III study among patients with coeliac disease with persistent symptoms despite GFD is ongoing[60] (TABLE 3). The inverse dose response feature of the larazotide data is poorly understood. However, the currently accepted hypothesis is that, as larazotide is a peptide, it is easily digested into smaller peptide fragments, some of which could have partial antagonist activity[59]. At different doses, the ratio of intact larazotide to larazotide fragments could differ resulting in different overall drug effect. Despite being the only drug advanced to phase III

clinical trial, issues concerning larazotide's suitable dosing and effects on small-bowel mucosa[61] (TABLE 3) remain to be solved.

Peptidase therapy

Gluten is immunogenic because its component proteins are only partially digested by mammalian gastrointestinal proteases[13]. Oral enzymatic therapy, a widely investigated therapeutic approach in coeliac disease, is focused on inactivating immunogenic gluten peptides in the human gastrointestinal tract by peptidase supplementation, minimizing the gluten peptides reaching the small intestine. Several proteases, peptidases and microorganisms have proven capacity to degrade gluten [62,63]. Some of them completely abolish the effects of the main immunogenic gluten peptides when tested *ex vivo* using T-cell assays from intestinal biopsy samples or peripheral blood mononuclear cells of patients with coeliac disease[64,65]. However, peptidases have to meet the following requirements before their use in clinical practice: demonstrate stability at the inhospitable conditions in the upper gastrointestinal tract (low pH, resistant to host proteases); efficiently degrade the multiple immunogenic peptides present in gluten in the stomach; and not have a deleterious effect in the patient[66].

The most studied enzymes with the ability to carry out this process belong to the prolyl endopeptidase family (PEP). Although PEPs are widely expressed in both mammals and microorganisms, the levels in humans are insufficient for detoxifying gluten peptides[66]. PEPs from a variety of bacteria and fungi including *Aspergillus niger*, *Flavobacterium meningosepticum*, *Myxococcus xanthus* and *Sphingomonas capsulata* are able to degrade gluten peptides both *in vitro* and *in vivo*[66].

Specifically, AN-PEP derived from *Aspergillus niger* degrades gluten at low pH and is resistant to digestion by pepsin as demonstrated in a human gastrointestinal tract model system[67]. AN-PEP was shown to be well-tolerated in a randomized double-blind, placebo-controlled pilot study including altogether 16 AN-PEP treated patients with coeliac disease consuming gluten[68] (TABLE 2). AN-PEP also significantly enhanced gluten digestion in the stomach of 12 healthy volunteers in a randomized, double-blind, placebo-controlled, cross-over study[69]. In a second randomized placebo-controlled crossover study, 18 subjects with self-reported gluten sensitivity but excluded for coeliac disease consumed 0.5 g gluten together with tablets containing AN-PEP or placebo. This study concluded that AN-PEP significantly degraded most gluten in the stomach before it entered the duodenum [49]. Whether AN-PEP prevents duodenal mucosal damage during gluten challenge remains to be answered in a phase II study[70] (TABLE 3).

STAN1, a cocktail of microbial enzymes commonly used in food supplements with modest gluten detoxification capacity[71], has also been tested in a phase II clinical trial[72] (TABLE 3). This randomized double-blind placebo-controlled study evaluated the effect of STAN1 supplementation on 38 GFD-treated but seropositive patients with coeliac disease with the primary outcome being negative seroconversion or a decrease of anti-TG2 antibody levels. However, according to a conference Abstract the primary outcome was not met as no differences were found in serology between the placebo group and the patients treated with STAN1[73], although the final results await to be published.

ALV003, or latiglutenase, is the most extensively investigated enzyme mixture in human trials. ALV003 is an orally administered mixture of two gluten-degrading proteases active in the acidic environment of the stomach: ALV001, a glutamine-specific cysteine endoprotease

from germinating barley seeds, and ALV002 a modified recombinant-version of a PEP from *Sphingomonas capsulate*[74]. After a phase I clinical trial showing drug tolerance and stability[75], a phase IIa clinical trial was performed in 20 adults with coeliac disease patients receiving ALV003 and 21 patients receiving placebo together with a daily gluten challenge[76] (TABLE 2). This study demonstrated that ALV003 seemed to attenuate gluten-induced small intestinal mucosal injury in patients in the context of a daily dose of up to 2 g gluten. However, gastrointestinal symptoms, headache and fatigue induced by gluten challenge did not differ between treatment arms[76]. A phase IIb, double-blind, placebo-controlled, dose-ranging study assessed the efficacy and safety of ALV003 in a large cohort of 494 GFD-treated patients with moderate or severe symptoms of coeliac disease. ALV003 did not improve histology or symptom scores when compared with placebo[77]. However, the study authors re-evaluated data of the same trial in a post-hoc analysis and found that seropositive patients with coeliac disease showed symptomatic improvement from ALV003 taken with meals[78]. Currently, two further phase II studies are underway (TABLE 3). Effects of ALV003 are evaluated during a 6-week gluten challenge study on histopathological parameters of treated patients with coeliac disease[79] and in a 6-month study to test the efficacy, safety and tolerability in symptomatic patients who are adherent to a GFD[80].

Protease supplementation seems a promising future therapy in coeliac disease and could exploit PEPs or other proteases. For instance, Kuma062 (PvP001 and PvP002) has shown capacity to degrade >99% of immunogenic gliadin peptides in gastric conditions in vitro[65] and it is currently in phase I clinical trial (TABLE 3). However, results from completed and published clinical trials do not yet support either enhanced clinical remission after peptidase supplementation or their use as a substitute for GFD. The main concern is the difficulty of degrading all the immunogenic sequences present on a daily gluten load. These enzymes

could improve the patients' response to low gluten exposure and reduce the incidence of non-responsive coeliac disease from inadvertent gluten ingestion showing potential as adjunctive therapy.

Anti-IL-15 treatment and other immune targets

IL-15 and its downstream signalling route are interesting drug targets as IL-15 is upregulated in both intestinal epithelial cells and lamina propria in active coeliac disease and is involved in key cellular processes in humans [24]. Results have been reported for AMG 714, which is a fully human monoclonal antibody that binds to all forms of IL-15 inhibiting their functions. AMG 714 has been tested in two randomized, double-blind, placebo-controlled phase IIa studies (TABLE 2). One study enrolled 64 GFD-treated patients with coeliac disease who were assigned to receive either 150 mg or 300 mg AMG 714 or placebo subcutaneously once every 2 weeks for a total of six doses[81]. The primary outcome was not met as AMG 714 did not prevent the small bowel mucosal injury during a gluten challenge of 2–4 g daily. However, despite the density of IELs increasing in all groups, in those receiving 300 mg of AMG 714 the increase was less pronounced. Moreover, of the clinical symptoms, diarrhoea was ameliorated with both doses of the drug. No serious adverse effects were reported during the study.

The same drug was tested in patients with RCDII in which the overexpression of IL-15 in the intestine has been postulated to contribute to the expansion of the aberrant IEL subpopulation characteristic for the condition[34]. During the trial, 19 patients received 8 mg/kg of AMG 714 and 9 patients placebo intravenously over 10 weeks for a total of seven doses[82]. The AMG 714 group did not differ from the placebo in the primary endpoint, which was the reduction of the proportion of aberrant intraepithelial lymphocytes from baseline . However, there was

a statistically significant difference in the proportion of patients who experienced at least one episode of diarrhoea per week during the study; in the AMG 714 group the proportion of these patients decreased and in the placebo group increased. In the AMG 714 group 26% and in the placebo group 11% of patients experienced serious adverse events including tuberculosis, pneumococcal infection and cerebellar syndrome. A phase IIb study has been started to evaluate the safety and effects of AMG 714 (called PRV-015 here) on symptoms among patients with non-responsive coeliac disease[83] (TABLE 3).

IL-15 signalling involves three different receptor chains: the common cytokine receptor γ chain shared by five other γ chain cytokines, including IL-21, the IL-2–IL-15 receptor β (IL-15Rβ) shared with IL-2 and a third receptor subunit IL-15Rα specific for IL-15[84]. These receptor chains have also been investigated as potential therapeutic targets in coeliac disease. BNZ-2 is a peptide that binds the common γ chain and has been shown specifically to inhibit IL-15 and IL-21-mediated activation of human intraepithelial cytotoxic T cells [85]. Given that a considerably greater proportion of patients with active CeD overexpress both IL-15 and IL-21 instead of either one of the cytokines selectively[85], BNZ-2 is an interesting future therapeutic candidate in coeliac disease. The humanized Mik-β-1 monoclonal antibody (Hu-Mik-β-1), on the other hand, targets the cytokine receptor subunit IL-2–IL-15Rβ[84]. Its efficacy has been investigated in patients with RCD in a phase I trial completed in 2019[86] (TABLE 3), but the results have not yet been published.

Following IL-15 binding to the receptor, Janus kinase (Jak)1 and Jak3 are activated, transducing the signal further[84] and, interestingly, a Jak2/3 inhibitor tofacitinib and Jak1/2 inhibitor ruxolitinib are in clinical use for other disorders. Of these inhibitors, tofacitinib has demonstrated some beneficial effects in transgenic mice overexpressing human IL-15, which

mimics RCD[87], and is currently being tested for RCDII in an ongoing phase II clinical trial[88] (TABLE 3). Other immune-mediated approaches for coeliac disease therapies already in clinical trials include, for instance, strategies to block T-cell homing to the intestine by CCR9 receptor antagonist CCX282-B[89] and by α4β7 integrin antagonist PTG-100[90] and to interfere with antigen presentation to HLA class II by cathepsin S inhibitors[91,92] (TABLE 3). Moreover, based on experimental evidence reducing inflammation by suppressing gluten-activated T cells by anti-CD3-antibodies[93] and targeting disease-relevant peptides presented on HLA-molecules on antigen presenting cells by T cell receptor-like monoclonal antibodies[94] could be viable means in the treatment of coeliac disease. However, the efficacy of these strategies in coeliac disease remains to be determined.

Gluten tolerization

Approaches that restore immune tolerance to gluten in people with coeliac disease are highly desirable as they target the underlying cause of disease and could have potential to enable the safe resumption of dietary gluten. Antigen-specific approaches are particularly attractive as they target the peripheral effector and/or memory autoreactive T cells without impairing broader immune function[95] (FIGURE 2). Such interventions might achieve their effect through two non-mutually exclusive mechanisms: clonal inactivation (unresponsiveness) and deletion of the pathogenic population by restimulation-induced cell death, and immune deviation via the generation of a regulatory T (Treg) cell population[95,96]. Treg cells can induce a tolerogenic phenotype in other T cells, a process known as infectious tolerance or linked suppression that can help to promote tolerance to a broader range of epitopes distinct from those inducing the Treg cells[96]. Several findings support the feasibility of antigen-specific tolerogenic approaches in coeliac disease. A major advance in coeliac disease, in contrast to other autoimmune diseases, is the detailed knowledge of the driving antigen, the T

cell epitopes in gluten, that triggers pathogenic T cells[20]. Furthermore, gluten-specific T cells are a stable population that maintain their T cell specificity and can persist for decades in the blood and intestine of patients with coeliac disease[97].

The most widely studied tolerogenic therapy for coeliac disease, Nexvax2, is a therapeutic vaccine composed of three gluten peptides encompassing five HLA-DQ2-restricted epitopes frequently recognized by gluten-specific T cells[22] (TABLE 2). In two phase I trials, with 82 and 36 patients with coeliac disease, intradermal administration of Nexvax2 initially caused symptoms including diarrhoea and nausea similar to those triggered by gluten ingestion but with successive dosing or with stepwise dose escalation symptoms were no different to those after placebo[98,99]. Nexvax2 substantially reduced recall T cell responses induced by oral gluten of 9 g daily to the epitopes in Nexvax2 and duodenal histology remained stable or trended towards improvement[98,99]. A striking serum cytokine level elevation dominated by IL-2 was measured 2–6 h post-injection of Nexvax2 and it correlated with the magnitude of symptoms, suggesting Nexvax2 was targeting disease-relevant intestinal T cells[100]. Having established positive clinical and immune modifying effects after Nexvax2 injection, a multicenter Phase II trial (RESETCeD) was conducted with the primary efficacy endpoint being protection against gluten-induced symptoms following double-blind crossover bolus food challenge with 6 g gluten or placebo[101]. The study was ceased after an interim analysis showed Nexvax2 did not provide statistically significant protection from gluten-induced symptoms[102]. Detailed efficacy findings are awaited. For patients in the placebo drug arm, oral gluten challenge induced strong gastrointestinal symptoms, primarily nausea and vomiting, in 61% and 44% respectively, compared to 6% and none after the placebo food challenge[103]. This suggests that suppression of immune responsiveness after Nexvax2 at the dose administered was insufficient to significantly modify digestive symptoms after a potent

gluten challenge. Resolving the relationship between gluten-specific immunity, objective endpoints such as histology and serology, and patient symptomatology remains an important challenge for the field.

Tolerizing immune-modifying particles containing gliadin (TIMP-GLIA) employ gliadin protein encapsulated in a polymeric nanoparticle[104]. Based on experimental data, after systemic administration, TIMP-GLIA are thought to be taken up by antigen presenting cells in the spleen and liver that process and cross-present a range of gliadin peptides in a tolerogenic manner. Injection of TIMP-GLIA into three mouse models of coeliac disease suppressed inflammatory T and B cell responses to gliadin recall and reduced gluten-dependent enteropathy[104]. A phase I study evaluating the safety, tolerability and pharmacokinetics of TIMP-GLIA[105] (TABLE 3) in 23 subjects is completed, and according to preliminary results some participants had drug-related adverse events whereas no serious adverse events were observed . A phase II, double-blind, randomized, proof-of-concept study with 34 patients with coeliac disease who received infusions of TIMP-GLIA or placebo and, starting one week later, consumed wheat gluten for 14 days, has been completed in August 2020[106] (TABLE 3). According to preliminary results of this phase II trial, patients treated with TIMP-GLIA showed a statistically significantly six-fold lower memory recall response of gliadin-specific T cells after gluten challenge of 6–12 g daily compared with placebo and there was a trend towards reduction in villous damage[107]. Further development of TIMP-GLIA is underway and another phase II study evaluating the optimal dosing of TIMP-GLIA infusion in GFD-treated coeliac disease patients during gluten challenge is planned[108] (TABLE 3).

Several additional antigen-specific approaches are also in the pipeline. One approach loads disease-relevant peptide–MHC complexes directly onto synthetic nanoparticles to promote regulatory T and B cells[109]. Another uses red blood cells coupled to gluten based on the premise that when circulating erythrocytes undergo apoptosis, a naturally tolerogenic process, the immune system recognizes the attached antigen and elicits a tolerogenic response[110]. KAN-101 is an investigational drug exploiting this technology that is being tested for safety and tolerability in a phase I clinical trial in patients with coeliac disease on GFD[111] (TABLE 3).

Parasitic helminths, including the human-infecting hookworm *Necator americanus*, have shown promise as modulators of mucosal inflammation in human illnesses such as Crohn's disease[112]. In a trial, 12 participants with coeliac disease inoculated with *N. americanus* undertook a 2-week oral gluten challenge (up to 3 g daily) and, compared to historical controls, had less mucosal deterioration and no rise in coeliac disease serology[113] (TABLE 2). However, in another 21-week double-blind, placebo-controlled phase Ib/IIa trial with 20 GFD-treated patients with coeliac disease, hookworm infection had no protective effect on the small-bowel mucosa during a gluten challenge of 16 g per day[50]. A phase Ib randomized, placebo-controlled study of *N. americanus* with gluten challenge was completed in 2019 and results are awaited[114] (TABLE 3).

TG2 inhibition

The key enzyme in coeliac disease pathogenesis, TG2 is a ubiquitously expressed protein with a multitude of functions including GTPase activity, cross-linking and capacity to catalyze posttranslational modifications by amine incorporation and deamidation[115]. Of these processes, the deamidation reaction is a crucial step in coeliac disease pathogenesis. The

catalytic mechanism of deamidation of gluten peptides involves the formation of a thioester intermediate between an active site cysteine residue in the catalytic core of TG2 and a glutamine side chain of the peptide, which reacts with water and leads to the transformation of the glutamine side-chain into a glutamic acid[116].

A number of preclinical proof-of-concept studies have revealed that inhibiting TG2 activity has several beneficial effects relevant for coeliac disease. Firstly, TG2 inhibition is able to block gliadin-induced proliferation of gliadin-specific T cells and prevent the increase of activated T cells in patient small bowel mucosal biopsy sample organ culture[117,118]. Moreover, inhibition of TG2 is able to modulate intestinal epithelial permeability functions *in vitro*[119,120]. In a newly established transgenic HLA-DQ8 mouse model overexpressing IL-15 in the gut epithelium and lamina propria, two different pharmacological TG2 inhibitors ERW1041E and CK805 prevented the development of gluten-induced villous atrophy[25].

One of the available TG2 inhibitors, ZED1227, is a highly specific orally active irreversible active site-directed TG2 inhibitor that has been shown in preclinical mouse experiments to be safe, effective and to reduce experimentally induced intestinal inflammation[121]. In a phase II trial, the efficacy of three doses (10, 50 and 100 mg) of ZED1227 are being tested for prevention of gluten-induced mucosal changes in individuals with well-controlled coeliac disease undergoing gluten challenge[122] (TABLE 3). The trial was completed in February 2020 and the results are awaited to be published.

Besides targeting the active site of TG2, a viable means to block TG2 activity is to exploit a redox mechanism involving the generation of an allosteric disulfide bond between two cysteine residues (Cys370 and Cys-371)[123]. Interestingly, disulfiram, a FDA-approved drug

for alcohol abuse, has been reported to inhibit TG2 activity *in vitro* likely by this mechanism,[124] raising further interesting possibilities for the future in terms of TG2 inhibition as a therapeutic strategy in coeliac disease.

Gluten sequestering

An interesting approach for coeliac disease therapy involves sequestering gluten in the intestinal lumen before it is digested into immunogenic peptides. This approach could be established with different compounds, including gliadin-targeting antibodies and polymeric binders.

Gliadin-reactive chicken yolk antibodies (AGY) have been shown to neutralize coeliac disease-inducing gliadin and reduce its absorption in mice[125]. In a phase I open-label, single-arm 6-week study conducted in 10 adults with coeliac disease on a GFD, oral AGY administration was shown to be safe and potentially associated with improved coeliac disease-related outcome measures[126] (TABLE 2). A larger randomized double-blind, placebo-controlled crossover trial aiming to enroll 149 GFD-treated patients with coeliac disease is currently ongoing, the primary outcome being patient-reported symptoms[127] (TABLE 3). As the use of yolk antibodies might be inefficient for clinical large-scale production, parallel recombinant antibody fragments in single-chain format directed against gliadin digest have been produced that could serve the same purpose[128].

The polymer BL-7010 or P(HEMA-co-SS), is a non-absorbable, high molecular weight molecule composed of hydroxyethylmethacrylate and sodium 4-styrene sulfonate that binds to α-gliadin[129]. Both *in vitro* and preclinical studies in mouse models of gluten sensitivity have demonstrated specific binding of BL-7010 to gliadin and reduction of its harmful

effects[130]. A phase I randomized double-blind, crossover placebo-controlled trial, with the objective to assess safety of BL-7010 and demonstrate lack of gliadin absorption in well-controlled patients with coeliac disease has been completed[131] (TABLE 3), but the results of the study have not been published.

Microbial therapy

The specific mechanisms by which microorganisms could participate in the protection against gluten-induced responses are broad and include the metabolism of the trigger antigen, enhancement of the intestinal barrier and modulation of adaptive and innate immune responses[31]. For instance, bacteria from our gastrointestinal tract have the capacity to degrade gluten *in vivo,* efficiently reducing its immunogenicity[132]. Several bacterial strains have demonstrated capacity to restore an increased intestinal permeability[133] and suppress adverse response to food components via nascent regulatory T cells inducing oral tolerance to food antigens in animal models [134]. Moreover, studies using experimental models of gluten sensitivity have shown that different bacteria including distinct Bifidobacteria reduce innate and adaptive immune pathways typically activated in coeliac disease[135,136]. Furthermore, orally administered gliadin-secreting bacteria have been reported to induce antigen-specific tolerance in an animal model[137]. However, the efficacy and safety of the tested strains in coeliac disease has to be further evaluated in clinical trials.

In a broad sense, microbial therapies can also be regarded to comprise probiotic supplementation although it is noteworthy that commercially available probiotics lack mechanistic evidence for their use in coeliac disease. Despite this limitation, probiotic supplementations have been explored in an increasing number of human trials for their possibly beneficial effects. In a double-blind, randomized, placebo-controlled study on 33

newly diagnosed pediatric patients with coeliac disease on GFD, *Bifidobacterium longum* CECT 7347 led to a reduction in immune markers (serum TNF, IgA in stool and peripheral CD3+ T cells) and reduction in the numbers of the Bacteroides fragilis group (P= 0·020) in stool [138]. Moreover, three studies explored the capacity of *Bifidobacterium breve* strains B632 and BR03 in double-blinded, placebo-controlled interventions in 40-49 children with coeliac disease [139-141]. Similarly, the investigators reported changes such as an increase of Actinobacteria and a re-establishment of the physiological Firmicutes/Bacteroidetes ratio in the intestinal microbiota and their metabolic capacity, and reduction of serum TNF in patients when probiotic was supplemented.

In a multicentre study, the probiotic formulation VSL#3 was tested in 45 symptomatic, adults with coeliac disease on GFD[142]. However, no differences in symptom severity and faecal microbiota were reported after probiotic supplementation. On the contrary, a double-blind, randomized placebo-controlled study performed in 109 patients with coeliac disease with irritable bowel syndrome-type symptoms according to the ROME III criteria such as recurrent abdominal pain or discomfort and changes in frequency or form of stool showed that a combination of lactic acid bacteria improved gastrointestinal symptoms and increased presumptive lactic acid bacteria, Staphylococcus and Bifidobacterium in the stool [143]. Also, an exploratory, randomized, double-blind, placebo-controlled study exploring the effects of *Bifidobacterium infantis* Natren Life Start in 22 adults with untreated active coeliac disease showed that this probiotic improved gastrointestinal symptoms and normalized immune markers, including reduction in αdefensin and Paneth cells in duodenal biopsy samples[144,145]. Coeliac disease was confirmed with duodenal biopsy at the end of the study in all patients. Research in this field is continuing and future studies will shed more light on the possible benefits of microorganisms as therapeutic strategy.

Prevention in the future?

Prevention of coeliac disease is a highly attractive goal and identification of environmental factors predisposing to the condition might provide targets for prevention. Two randomized controlled trials with 944 and 832 infants with a first-degree relative with coeliac disease, have been conducted to evaluate if early introduction of small quantities of gluten or delayed introduction of gluten reduced the risk of coeliac disease. However, neither of these strategies was effective in preventing coeliac disease[146,147]. Also, the possible role of infections in disease development, and especially viruses such as adenovirus, enterovirus, rotavirus and reovirus, might provide ways for prevention of coeliac disease[26,28-30], but this approach requires further research.

The idea that probiotics might prevent the development of coeliac disease has also been introduced. In a Swedish trial, 78 children on a gluten-containing diet with HLA-susceptibility for coeliac disease and positive IgA TG2 antibodies were randomly assigned to receive a probiotic (two *Lactobacillus* strains) or placebo for 6 months[148]. The investigators identified differences in T cell subsets between treatment arms and TG2-IgA levels decreased more substantially in the probiotic arm but coeliac disease developed in the same proportion of children in both groups. Furthermore, an overall exposure to probiotics during the first year of life has not been associated with a protective effect against the development of coeliac disease in genetically at-risk children[149]. A study in genetically susceptible infants 4 months and older is underway to evaluate the effects of GFD and probiotics during the first 3 years of life on the development of coeliac disease[150]. Despite being a target for investigations, prevention of coeliac disease remains currently elusive. However, increased future understanding of how genomic, immune, environmental and microorganisms–host

interactions conspire to cause coeliac disease, could make preventive measures to become realistic.

Outlook

Despite a range of challenges, the current treatment of coeliac disease with a GFD is well-tolerated and safe. Although gluten exclusion has been effective in improving symptoms and mucosal damage, strict adherence to the GFD is challenging. Ideally, new treatment modalities would provide greater efficacy than the current GFD, but have an equivalent safety profile.

To date, the tested drug candidates have generally proven safe and well-tolerated in clinical trials. Severe adverse events have only been reported in already very unwell patients with RCDII in the AMG 714 trial. In addition, many of the investigative therapies including larazotide, ALV003 and AMG 714, have had beneficial effects on gluten-induced symptoms but, with the exception of ALV003 in a single study, none of them have rigorously assessed attenuation of small intestinal mucosal injury. Understanding the effect of therapies on small bowel mucosal damage should be a focus in phase II and III clinical trials.

Current drug candidates in development are not able to replace a GFD, but are promising as an adjunctive therapy for patients experiencing symptoms despite following a strict diet. Yet, with adjunctive therapies, there is a possible risk to develop asymptomatic enteropathy due to inadvertent gluten exposure during the treatment, which should be considered. In addition, none of the novel treatment options apart from microbes have been tested in children, so their suitability to pediatric patients remains unresolved until further data are available.

Another issue to consider with potential novel treatments is their cost to the patient and the health care system. Although maintaining a strict GFD is more expensive than a gluten-containing diet, the novel drugs are likely to add to overall cost. Some of the tested treatment modalities, particularly antibodies such as AMG 714 and tofacitinib for instance, are likely to be expensive, which could restrict their use in uncomplicated disease. However, in patients with RCD, associated with increased morbidity and health care costs, these approaches might be more acceptable.

Conclusions

Several potential therapeutic targets based on emerging knowledge of coeliac disease pathogenesis have been identified and exploited in developing new drugs. The approaches under investigation encompass interfering with processing of gluten in the intestinal lumen, blocking gluten-induced adaptive or innate immune responses, and restoring immune tolerance to gluten. Research in these areas remains very active and clinical trials to test the efficacy of the novel therapies have already been completed or are still ongoing. The progression of new drug candidates towards phase III clinical trials underlines the need for reliable, non-invasive surrogate markers for gluten-induced small intestinal damage and effective patient-reported outcome measures. To replace GFD, the new drug should be able to prevent the development of the gluten-induced damage in the small bowel mucosa, but of the tested modalities to date, none has convincingly demonstrated such efficacy. Some drug candidates have alleviated symptoms and might therefore be useful as adjunctive therapy for patients experiencing symptoms despite a strict GFD. Preventing the onset of coeliac disease entirely would be the most beneficial and desirable way to tackle the disease, but more research is required to establish rational targets for disease prevention. For now, the GFD remains the gold standard treatment for coeliac disease. However, as new drug trials are

completed and reported in the near future, data on promising treatment strategies are likely to emerge.

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Competing interests:

L.K. reports personal fees for lectures from Finnish Coeliac Society outside the submitted work and participation to AMG 714 trial. D.A.L. is the Medical Director for Takeda Pharmaceuticals. J.T.-D. is an inventor of patents pertaining to the use of gluten-derived T cell epitopes for use in CeD therapeutics and was an investigator in the Nexvax2 Phase II trial. The other authors declare no competing interests.

BOX 1 | What are gluten and the gluten-free diet?

-Gluten is the major protein component of wheat and is the substance remaining when wheat flour is washed to remove the starch

-Due to its unique viscoelastic properties, gluten is essential for dough formation and is an important and ubiquitous ingredient in food industry

-Although strictly speaking gluten is only present in wheat, the term also commonly refers to the proteins of similar composition in rye (secalins), barley (hordeins) and oats (avenins)

-Gluten and its counterparts are proline and glutamine rich proteins (e.g. prolamins) and the high proline content makes them fairly resistant to gastrointestinal digestion

-Wheat gluten is a complex mixture of alcohol soluble gliadins and alcohol-insoluble glutenin

-Gliadins are divided up into α- gliadins, γ-gliadins and ω- gliadins, and glutenin into high-molecular-mass and low-molecular-mass glutenins

- Patients with coeliac disease respond to a broad range of gluten peptides, but only a limited number of immunodominant peptides (such as 33-mer) elicit immunological responses in most patients

-Gluten-free diet refers to exclusion of wheat, rye and barley and products containing these grains from the diet

-The suitability of oats to patients with coeliac disease remains controversial and recommendations about its inclusion/exclusion vary between countries. Oats as a part of GFD is accepted in for instance in Scandinavian countries, the United Kingdom, the United States and Canada but not recommended in Australia and New Zealand

-Corn, buckwheat, rice, quinoa, soy, sorghum, tapioca and flax are naturally gluten-free grains and therefore suitable for patients with coeliac disease

BOX 2 | Possible challenges with gluten-free diet.

Ensuring gluten avoidance

- High cost

- Poor palatability

- Risks when dining out

- Inadequate or unclear food and drug labelling

- Inaccurate dietary information

- Difficulty to avoid inadvertent gluten exposure

Maintaining well-balanced diet

- Overall versatility of the diet

- Insufficient fibre and whole grain intake

- Weight control

- Prevention of disordered eating

Food-related social situations

- Peer and/or cultural pressures

- Anxiety and social isolation

- Impaired quality of life

- Unnecessary restrictions in everyday life

Table 1 | Main outcome measures and their difficulties in coeliac disease treatment trials.

Outcome	Methods of measurement	Challenges
Histology	Categorical and quantitative reporting of morphology (e.g. Marsh–Oberhuber classification and villous height:crypt depth ratio), and IEL count	Invasive; technical issues with biopsy orientation and assessment; highly limited sampling of villi; individual variation in responsiveness of mucosal damage to gluten exposure and therapy.
Serology	TG2, endomysial and deamidated gliadin peptide antibodies	Varying quality of commercial assays; unknown prognostic significance; slow response to gluten.
Clinical outcomes	Reported by patients or clinicians. Symptoms, disease activity, health-related quality of life	Wide variety of questionnaires and tools; natural fluctuation of symptoms; subjectivity; placebo and/or nocebo effects and lack of specificity to gluten exposure; clinically meaningful change is unclear.
Potential activity markers	Cytokine release including IL-2; gluten-reactive T cells; video capsule endoscopic small bowel assessment; mucosal TG2 IgA deposits; molecular histology and/or mucosal genetic biomarkers and peripheral markers of intestinal damage including intestinal fatty acid-binding protein	More data needed, variable sensitivity, specificity and responsiveness; unclear relationship to traditional histology and symptoms.

Abbreviations: IEL, intraepithelial lymphocyte; TG2, transglutaminase-2.

Table 2 | Published clinical trials on coeliac disease treatment and their main findings.				
Therapy	**Targeted mechanism**	**Trial phase**	**Number of participants**	**Main findings**
AGY	Gluten sequestration	Phase I	10	Safe for patients with coeliac disease. [126].
ALV003 (latiglutenase)	Peptidase therapy	Phase I	81	Well-tolerated by patients with coeliac disease and healthy individuals, degrades gluten in the stomach[75].
		Phase II	41	Attenuates gluten-induced mucosal injury in coeliac disease[76].
		Phase II	494	No improvement on histology and symptom scores in symptomatic coeliac disease when compared with placebo[77].
		Phase II	398	In symptomatic seropositive patients with coeliac disease, dose-dependent reduction in the severity and frequency of symptoms[78].
AN-PEP	Peptidase therapy	Phase I	16	Well-tolerated by patients with coeliac disease[68].
		NA	18[49] 12[69]	Substantially enhances gluten digestion in the stomach of healthy volunteers and gluten-sensitive individuals[49,69].
AMG 714	Anti-IL-15 therapy	Phase II	64	No effect on gluten-induced small bowel mucosal damage, diarrhoea was ameliorated, no serious adverse effects[81].
		Phase II	28	In RCDII, no effect on the number of aberrant intraepithelial lymphocytes, decrease in diarrhoea episodes [82].
Larazotide (AT-1001)	Blocking epithelial permeability	Phase I	21	Well-tolerated by patients with coeliac disease during gluten challenge[56].
		Phase II	86[57] 184[58] 342[59]	No effect on intestinal permeability, but low doses had beneficial effects on gastrointestinal symptoms[57-59].
Necator americanus	Gluten tolerization	Phase I and II	12	Well-tolerated by patients with coeliac disease, less mucosal

inoculation				deterioration and no rise in coeliac disease serology after gluten challenge compared with historical controls[113].
		Phase II	20	No protection for duodenal mucosa during a gluten challenge[50].
Nexvax2	Vaccine and tolerization	Phase I	82[98] 36[99] 82[100]	Well-tolerated with stepwise dosing, initial elevation of serum IL-2 followed by reduced T cell response to Nexvax2 peptides, no adverse effect on small bowel histology[98-100].
		Phase II	146	NCT03644069 trial discontinued as protection from symptoms induced by gluten was not greater than by placebo[102].
All trials in adults. Abbreviations: AGY, anti-gliadin immunoglobulin class Y; AN-PEP, *Aspergillus niger* prolyl endopeptidase; CeD, celiac disease; NA, not applicable; RCDII, refractory coeliac disease type II; TG2, transglutaminase-2.				

Table 3 | Ongoing or yet to be reported clinical trials for novel celiac disease drugs[a].

Therapy	Targeted mechanism	Study population	Outcome measures[b]	Clinical trial code
Phase III				
Larazotide (AT-1001)	Blocking epithelial permeability	GFD-treated but symptomatic patients with coeliac disease	Reduction of gastrointestinal symptoms (coeliac disease PRO Abdominal Domain scores)	NCT03569007
Phase II				
AGY	Gluten sequestering	GFD-treated but symptomatic patients with coeliac disease	Coeliac-related symptoms	NCT03707730
AMG 714 (PRV-015)	Anti-IL-15 therapy	GFD-treated but seropositive and symptomatic patients with coeliac disease	Symptoms (celiac disease PRO questionnaire), IELs, adverse events, PRV-015 concentrations and antibodies.	NCT04424927
AN-PEP	Peptidase therapy	GFD-treated patients with coeliac disease, gluten challenge	Gluten reactive Tcells, immunophenotype of lymphocytes, symptoms	NCT00810654
CCX282-B	CCR9 antagonist	GFD-treated patients with coeliac disease, gluten challenge	Vh:Cd, mucosal inflammation, coeliac serology and symptoms	NCT00540657
Larazotide (AT-1001)	Blocking epithelial permeability	Untreated patients with coeliac disease	Vh:Cd, safety and tolerability, symptoms (CeDARS, CGA) and quality of life (PWBI, SF-12v2), inflammatory markers	NCT00620451
Latiglutenase (IMGX003, ALV003)	Peptidase therapy	GFD-treated but symptomatic patients with coeliac disease	Severity of symptoms, health-related quality of life (PGI-I and PGI-S, ICDSQ, SF-12)	NCT04243551
		GFD-treated patients with coeliac disease, gluten challenge	Vh:Cd, gastrointestinal symptom severity	NCT03585478

STAN1	Peptidase therapy	GFD-treated but seropositive patients with coeliac disease	TG2Ab, EmA, coeliac disease rapid test, symptoms, small-bowel morphology	NCT00962182
TIMP-GLIA (CNP-101, TAK-101)	Gluten tolerization	GFD-treated patients with coeliac disease, gluten challenge	IFN-γ spot forming units, Vh:Cd, IELs, gliadin-specific T cell proliferation and cytokine secretion, gut-homing $CD4^+$, $CD8^+$ and γδ cells, IL-2, drug concentration and antidrug antibodies, symptoms (CDSD), adverse events	NCT03738475, NCT04530123
Tofacitinib	JAK inhibition	Patients with RCD II	Aberrant IELs, small-bowel histology, symptoms (BSFS, GSRS, coeliac disease PRO, CDSD), quality of life (EQ-5D-5L), safety and immunological changes	2018-001678-10
ZED1227	TG2 inhibition	GFD-treated patients with coeliac disease, gluten challenge	Small-bowel mucosal morphology, inflammation, PROs, serology, adverse events, plasma concentration and metabolites, safety and tolerability	2017-002241-30
Phase I				
BL-7010	Gluten sequestering	GFD-treated patients with coeliac disease	Adverse events, safety and tolerability, plasma levels of BL-7010	NCT01990885
Necator americanus inoculation	Gluten tolerization	GFD-treated patients with coeliac disease, gluten challenge	Vh:Cd, IELs, symptoms (Celiac Symptom Index), quality of life (Celiac-Quality of Life Score), TG2Ab	NCT02754609
Hu-Mik-β-1	Cytokine receptor antibody	Patients with coeliac disease	Safety	NCT01893775
KAN-101	Antigen specific immune tolerance	GFD-treated patients with coeliac disease	Adverse events, pharmacokinetics	NCT04248855
Kuma062 (PvP001 and PvP002)	Gluten degradation	Healthy individuals and patients with coeliac disease	Adverse events, safety, tolerability, pharmacokinetics, gluten degradation activity	NCT03701555
PTG-100	α4β7 integrin antagonist	Patients with coeliac disease, gluten challenge	Vh:Cd, TG2Ab, DGPAb, $CD3^+$ cells, CSI, immune modulators	NCT04524221

RO5459072	Cathepsin S inhibitor	GFD-treated patients with coeliac disease, gluten challenge	Number of gliadin specific T-cells, adverse events, coeliac serology, LMR, pharmacokinetics	NCT02679014
TIMP-GLIA (CNP-101)	Gluten tolerization	GFD-treated patients with coeliac disease	Adverse events, pharmacokinetics	NCT03486990

. [a]On 24th September 2020, Clinicaltrials.gov and EU Clinical trials register. [b]The main outcome of the studies is listed first. Abbreviations: AGY, anti-gliadin immunoglobulin class Y; AN-PEP, *Aspergillus niger* prolyl endopeptidase; BSFS, Bristol stool form scale; CDSD, Celiac Disease Symptom Diary; CeDARS, Celiac Disease Activity Rating Score; CGA, Clinician Global Assessment; CSI, Celiac Symptom Index; DGPAb, deamidated gliadin peptide antibodies; EmA, endomysial antibodies; EQ-5D-5L, Euro Quality of Life five-dimensional questionnaire; GFD, gluten-free diet; GSRS, Gastrointestinal Symptom Rating Scale; Hu-Mik-β-1, Humanized Mik-Beta-1 monoclonal antibody; ; ICDSC, Impact of Celiac Disease Symptoms Questionnaire; IEL, intraepithelial lymphocyte; JAK, janus kinase; PGI, Patient Generated Index; LMR, lactulose to mannitol ratio; PRO, patient-reported outcome; PWBI, Psychological Well Being Index; RCD, refractory coeliac disease; SF-12, 12-Item Short Form Survey; TG2Ab, transglutaminase-2 antibodies; Vh:Cd, villous high-crypt depth ratio.

FIG. 1 | The pathogenesis of coeliac disease and investigational approaches that have been tested as future treatments. Gluten is highly resistant to degradation by gastrointestinal enzymes and thus fairly long peptides persist in the intestinal lumen. Thus, treatment modalities that operate to sequester gluten prior to its digestion into harmful peptides (1) or further proteolysis of the immunogenic peptides (2) might be effective. The peptides traverse through the intestinal epithelial layer and therefore blocking epithelial permeability has been studied as a drug target in coeliac disease (3). During the pathogenetic process, relevant gliadin peptides are deamidated by transglutaminase 2 (TG2) thereby increasing their binding affinity to celiac disease-predisposing HLA-DQ2 and HLA-DQ8 molecules on antigen-presenting cells (APCs). TG2 inhibitors have therefore emerged as candidate drugs for coeliac disease (4). The HLA-bound peptides are presented to gluten specific T cells that become activated and start secreting proinflammatory cytokines such IFN-γ and IL-21. The gluten-specific T cells also communicate with B cells that differentiate into plasma cells producing antibodies against gluten peptides and TG2. Regaining tolerance to gluten by deleting effector T cells or inducing regulatory T cells has been investigated as another therapeutic option (5). Coeliac disease pathogenesis also involves an innate immune response characterized by upregulation of IL-15, which promotes intestinal epithelial cell damage. Blocking IL-15-mediated effects by antibodies has been investigated in clinical trials (6). Various microorganisms have been suggested to modulate relevant immune responses in coeliac disease, and the possible benefit of probiotics to improve intestinal homeostasis has been studied (7). Abbreviations: AGY, anti-gliadin immunoglobulin class Y; AN-PEP, *Aspergillus niger* prolyl endopeptidase; HLA, human leukocyte antigen; IEL, intraepithelial lymphocyte; TCR, T-cell receptor.

FIG 2. | Proposed mechanisms for gluten tolerization strategies. Current experimental immunotherapies aim to directly or indirectly target gluten-specific T cells and/or antigen presenting cells to modify the immune response to gluten. Tolerance may be acquired by rendering pro-inflammatory gluten-specific T cells non-reactive or by deleting them. It may also be promoted by the induction and expansion of functional regulatory T cells (Tregs) that can inhibit effector T cells, generate linked suppression of naïve T cells that have specificity for gluten and induce regulatory B cells (B regs). These mechanisms may act in concert however the optimal combination necessary for the induction of durable and clinically relevant tolerance to gluten remains unknown.

Fig 1.

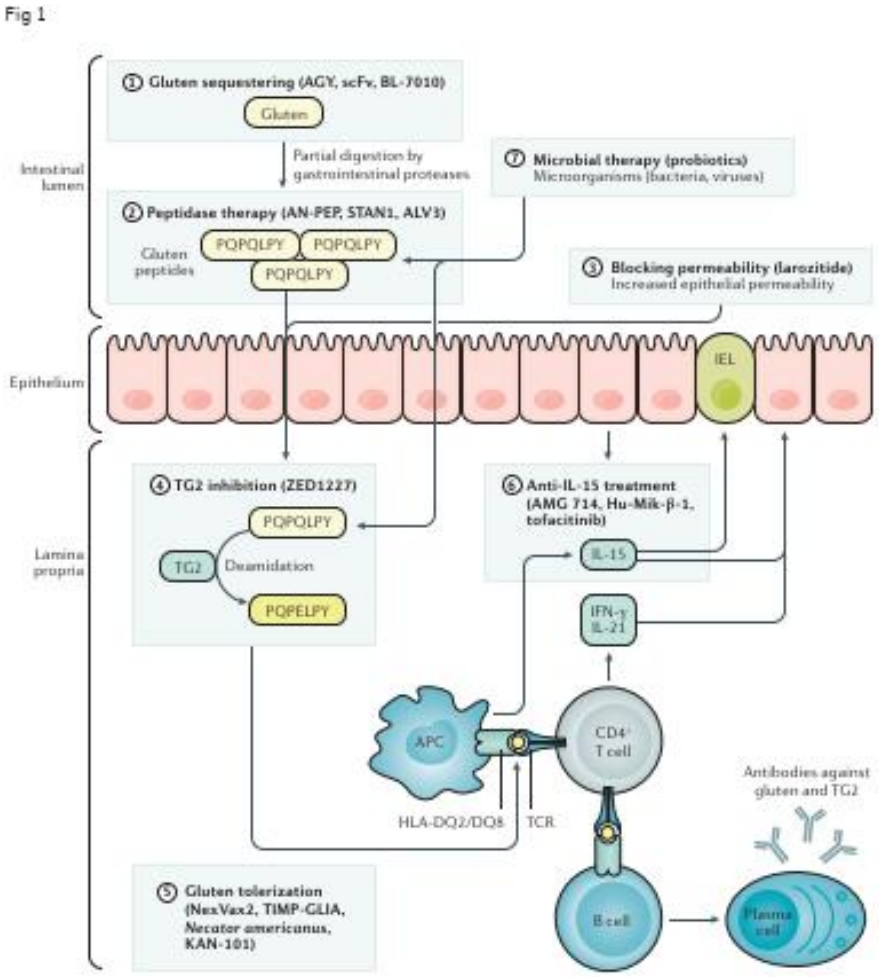
Fig 1
Intestinal lumen
Epithelium
Lamina propria
1 Gluten sequestering (AGY, scFv, BL-7010)
Gluten
Partial digestion by gastrointestinal proteases
2 Peptidase therapy (AN-PEP, STAN1, ALV3)
Gluten peptides
PQPQLPY
PQPQLPY
PQPQLPY
7 Microbial therapy (probiotics)
Microorganisms (bacteria, viruses)
3 Blocking permeability (larazotide)
Increased epithelial permeability
IEL
4 TG2 inhibition (ZED1227)
PQPQLPY
TG2
Deamidation
PQPELPY
5 Anti-IL-15 treatment (AMG 714, Hu-Mik-β-1, tofacitinib)
IL-15
IFN-γ
IL-21
APC
CD4+ T cell
HLA-DQ2/DQ8
TCR
Antibodies against gluten and TG2
B cell
Plasma cell
5 Gluten tolerization (NexVax2, TIMP-GLIA, Necator americanus, KAN-101)

Fig. 2.

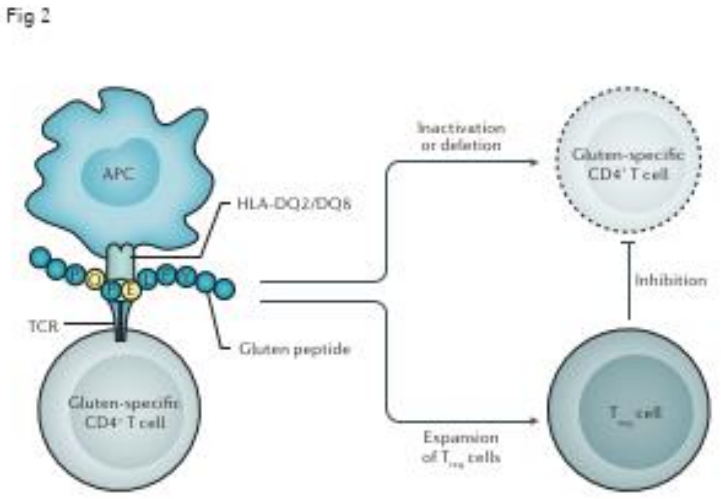
Fig 2
APC
HLA-DQ2/DQ8
TCR
Gluten peptide
Gluten-specific CD4+ T cell
Inactivation or deletion
Gluten-specific CD4+ T cell
Inhibition
Expansion of Treg cells
Treg cell