

# Celiac Disease

## A Challenging Disease for Pharmaceutical Scientists

**Journal Article****Author(s):**

Matoori, Simon; Fuhrmann, Gregor; [Leroux, Jean-Christophe](#) 

**Publication date:**

2013-03

**Permanent link:**

<https://doi.org/10.3929/ethz-b-000064569>

**Rights / license:**

[In Copyright - Non-Commercial Use Permitted](#)

**Originally published in:**

Pharmaceutical Research 30(3), <https://doi.org/10.1007/s11095-012-0951-x>

# Celiac Disease: A Challenging Disease for Pharmaceutical Scientists

Simon Matoori · Gregor Fuhrmann · Jean-Christophe Leroux

Received: 23 September 2012 / Accepted: 30 November 2012 / Published online: 11 December 2012  
© Springer Science+Business Media New York 2012

**ABSTRACT** Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten-containing grains that affects ~1% of the white ethnic population. In the last decades, a rise in prevalence of CD has been observed that cannot be fully explained by improved diagnostics. Genetic predisposition greatly influences the susceptibility of individuals towards CD, though environmental factors also play a role. With no pharmacological treatments available, the only option to keep CD in remission is a strict and permanent exclusion of dietary gluten. Such a gluten-free diet is difficult to maintain because of gluten's omnipresence in food (e.g., additive in processed food). The development of adjuvant therapies which would permit the intake of small amounts of gluten would be desirable to improve the quality of life of patients on a gluten-free diet. Such therapies include gluten-degrading enzymes, polymeric binders, desensitizing vaccines, anti-inflammatory drugs, transglutaminase 2 inhibitors, and HLA-DQ2 blockers. However, many of these approaches pose pharmaceutical challenges with respect to drug formulation and stability, or application route and dosing interval. This perspective article discusses how pharmaceutical scientists may deal with these challenges and contribute to the implementation of novel therapeutic options for patients with CD.

S. Matoori  
Department of Chemistry and Applied Biosciences  
ETH Zurich Institute of Pharmaceutical Sciences  
Wolfgang-Pauli-Str. 10, HCI  
8093 Zurich, Switzerland

G. Fuhrmann  
Department of Chemistry and Applied Biosciences,  
ETH Zurich Institute of Pharmaceutical Sciences  
Wolfgang-Pauli-Str. 10, HCI | J 398  
8093 Zurich, Switzerland

J.-C. Leroux (✉)  
Department of Chemistry and Applied Biosciences,  
ETH Zurich Institute of Pharmaceutical Sciences  
Wolfgang-Pauli-Str. 10, HCI H 301  
8093 Zurich, Switzerland  
e-mail: jleroux@ethz.ch

**KEY WORDS** adjuvant therapy · autoimmune disorder · celiac sprue · drug formulation · polymeric drug

## ABBREVIATIONS

APC	antigen-presenting cell
CD	celiac disease
GFD	gluten-free diet
GI	gastrointestinal
HLA	human leukocyte antigen
IEL	intraepithelial lymphocyte
IgA	immunoglobulin A
IL-15	interleukin-15
P(HEMA-co-SS)	poly(hydroxyethylmethacrylate-co-styrene sulfonate)
PEP	prolyl endopeptidase
TfR	transferrin receptor
TG2	transglutaminase 2

## INTRODUCTION

Celiac disease (CD) is defined as “a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals” (1). Nonetheless, CD is a systemic disease with a wide variety of clinical symptoms (2,3). A recent study based on serologic testing found a prevalence of up to 1% among the white ethnic population (4). CD prevalence has been on the rise for many decades, with a five-fold increase reported in the United States since the 1970s. Improved diagnostic methods do not fully account for this increase (5). In some populations, such as in type I diabetics (at least 10%), the prevalence is even higher (6). As CD is seen as a growing and serious health problem, developing therapeutic strategies against CD may have a profound scientific and clinical impact. In this perspective article, the pathogenesis and

diagnostic methods for CD are briefly reviewed and the pharmaceutical challenges associated with future therapeutic options are critically discussed.

## **PATHOGENESIS OF CD**

CD is triggered by gluten, which is a mixture of digestion-resistant proteins found in wheat, barley, and rye (7). Gluten is a very common nutritional component in Western countries, with an average intake of approximately 20 g per day per person (8). It consists of polymeric (glutenins) and monomeric (gliadins) protein fractions that possess a high immunogenic (i.e., activation of adaptive immunity) or toxic (i.e., activation of innate immunity) potential in genetically predisposed individuals (8,9). Some gliadin segments are highly stable towards degradation by intraluminal proteases and intestinal brush-border membrane enzymes due to their high proline and glutamine content (10). Consequently, large peptide fragments of gluten remain intact in the duodenal lumen, and subsequently cross the epithelium para- and transcellularly to reach the *lamina propria* (11). Paracellular permeability is linked to compromised tight junction integrity where gluten is thought to stimulate the release of the intestinal peptide zonulin, an agent that reversibly opens tight junctions (12). The transcellular pathway involves secretory immunoglobulins A (IgA), which bind gliadin peptides, and are subsequently internalized by the transferrin receptor (TfR, CD71) of epithelial cells (13). The peptides are then deamidated by tissue transglutaminase 2 (TG2), an enzyme catalyzing the hydrolysis of glutamine to glutamic acid residues (14). TG2-mediated deamidation introduces negative charges into the glutamine-rich gliadin peptides such that their avidity to human leukocyte antigen (HLA)-subtypes DQ2 and DQ8 of antigen-presenting cells (APCs) is increased (15). APCs present the antigen to naïve gluten-reactive CD4<sup>+</sup> T cells and activate them. These T lymphocytes secrete proinflammatory cytokines (e.g., INF $\gamma$ , TNF $\alpha$ ) and induce the characteristic antibody response by activation of gluten- and TG2-reactive B cells (8,16). The role of anti-TG2 autoantibodies in the pathogenesis of CD is not well understood. Pathogenic effects on enterocytes and on the intestinal epithelial barrier were described but remain of uncertain clinical relevance (16). However, immunodeposits of autoantibodies against TG2 and the TG-isoform of the epidermis TG3 (due to cross-reactivity or epitope spreading) were found in skin lesions of an extraintestinal manifestation of CD, dermatitis herpetiformis (16). The innate immunity is also implicated in the pathogenesis of CD (17). Enterocytes, dendritic cells, and macrophages secrete proinflammatory cytokines such as interleukin-15 (IL-15), which mediate abnormal cellular cytotoxicity by intraepithelial

lymphocytes (IEL). Activation of IELs, and in addition secretion of matrix metalloproteases by fibroblasts and *lamina propria* mononuclear cells result in histologically detectable lesions of the intestinal epithelium (8,16,18). Moreover, IL-15 in combination with retinoic acid prevents the generation of regulatory T cells (19).

## **SYMPTOMS AND DIAGNOSTICS**

CD is a systemic disease associated with symptoms of wide variety and intensity (1). Symptomatic CD patients present with intestinal and/or extraintestinal symptoms (anemia, osteoporosis, dermatitis herpetiformis) (1). Intestinal symptoms are further classified in typical malabsorption signs (e.g., weight loss, diarrhea, steatorrhea) and abdominal symptoms without malabsorption (e.g., abdominal pain, constipation, vomiting) (1,20). Asymptomatic forms do not show clinical manifestations even though they are serologically and histologically positive for CD (1,16). Potential CD cases exhibit a normal small intestinal histology but they are serologically positive for CD and at an elevated risk to develop the disease (1). Following the celiac iceberg model, it is assumed that typical CD cases are less prevalent than asymptomatic and potential forms (21). As a result, the ratio of diagnosed to undiagnosed CD cases is low, ca. 0.2 (4). Gluten also elicits adverse effects separate from CD such as IgE-mediated wheat allergy or non-celiac gluten sensitivity, a highly prevalent (up to 6%) condition in which gluten intake is associated with an increase in markers of the innate immune response and symptomatic manifestations (e.g., abdominal pain, behavioral changes, bone or joint pain and weight loss) even though CD is diagnostically ruled out (20,22). Genetic factors contribute to the risk of developing CD. More than 90% of CD patients express HLA-DQ2; the others express HLA-DQ8 (8). In view of the high frequency of HLA-DQ2 in controls (about 30%), HLA-DQ2 or DQ8 genes are necessary but not sufficient for the development of the disease (23,24). The HLA- and non-HLA-genes identified to modulate the probability of developing CD contribute around 50% to the disease risk (16). Moreover, monozygotic twins have a concordance rate of 75% to develop CD (16). Both factors support the influence of environmental factors involved in CD. A detailed discussion of the genetic aspects of CD is available in reference (25).

Tests to detect CD-specific antibodies are widespread because they are easy-to-use and non-invasive. They include detection of IgA-type anti-TG2, anti-deamidated gliadin and anti-endomysial antibodies (26). According to the *American Gastroenterological Association*, a diagnosis of CD relies on a positive antibody test and on a positive small-bowel mucosal biopsy upon gastrointestinal (GI) endoscopy (Medical

Position Statement 2006). However, the diagnosis of CD without biopsy is permitted in certain cases by the new guidelines of the *European Society for Paediatric Gastroenterology Hepatology and Nutrition* (27). Histologic characteristics of active CD include inflammation of the intestinal mucosa, increased IEL density, crypt hyperplasia, and in most cases villous atrophy (26). As HLA typing has a negative predictive value of almost 100%, it is a cost-effective alternative to exclude the diagnosis of CD in potential and asymptomatic cases (26).

## PREVENTION AND TREATMENT

With no effective pharmacological treatments available, preventive measures based on a better understanding of disease-influencing environmental factors may help reduce the incidence of CD. However, clear evidence on how prevention methods could reduce the prevalence of CD is lacking and clinical trials are still ongoing. For a detailed description of the role of environmental factors and preventive measures, the reader is referred to reference (8). After diagnosis of CD, a complete and life-long exclusion of gluten from the diet has to be implemented to keep CD in remission. Although a gluten-free diet (GFD) is beneficial (e.g., due to symptomatic relief), it is expensive and difficult to maintain, especially when eating out or travelling and in situations of increased social pressure (8). Approximately one third of CD patients do not fully adhere to a GFD due to social or educational reasons (28), resulting in increased morbidity and mortality (29). Fifty percent of patients that fully adhere to a GFD do not achieve histological remission because of continued gluten-intake of around 5–50 mg of gluten per day (30). The reasons for this unconscious exposure include inadvertent dietary consumption due to gluten-contamination or presence of gluten as an additive in processed food. In addition, rules on “gluten-free” labeling of foodstuff varies between countries (8). The production of wheat devoid of immunogenic peptidic sequences could ease dietary restrictions in the future. Such wheat can be generated by fermentation or genetic modification. Problems associated with this approach include reduced baking quality, gluten-contamination, and continued immune response to rye and barley (Fig. 1) (8,30,31). Several pharmacological adjuvant therapies which would allow safe ingestion of small amounts of gluten are currently being explored (Table 1). They are discussed in more detail below.

### Desensitization

Repeated administration of select immunogenic gliadin peptides was shown to inhibit T cell proliferation and expression of proinflammatory cytokines (IL-2, IFN $\gamma$ ) in a

transgenic HLA-DQ2 mouse model (32). Nexvax2<sup>®</sup> (ImmuSanT) is a desensitizing vaccine consisting of three immunogenic gluten peptides from wheat, barley and rye, developed as an immunotherapeutic and prophylactic agent to restore gluten tolerance (33). In a phase I clinical trial (NCT00879749), Nexvax2<sup>®</sup> was weekly administered to HLA-DQ2-positive patients on a strictly GFD by intradermal injection for 3 weeks. Detection of INF $\gamma$ -producing Nexvax2<sup>®</sup>-specific T cells in several subjects confirmed the bioactivity of the vaccine (33). Even though vaccination against CD is the preferred option for CD patients when asked to rank alternatives to GFD (31), the periodicity of Nexvax2<sup>®</sup> injections may have a negative impact on compliance. Therefore, sustained release formulations composed of biodegradable polymers (e.g., aliphatic polyesters) such as microspheres or *in situ* forming implants could be explored in the future as alternatives to periodic injections. In phase Ib, intradermal injections with microneedles (e.g., BD Soluvia<sup>™</sup>, BD) are planned. Further challenges are a potential immune system activation with a disease flare in response to the vaccine (31), and the restriction to patients with HLA-DQ2. Another investigated means of desensitizing patients is hookworm-infection (34). CD patients were cutaneously inoculated twice with larvae of the hookworm *Necator americanus* in a phase Ib/IIa trial (NCT00671138), and subsequently underwent a wheat challenge for 5 days (16 g of gluten/day). No significant differences between infected and non-infected patients were seen with respect to histological, clinical, or inflammation-related parameters (34). Further analysis of the biopsy specimens, however, indicated that hookworm-infection lowered the production of proinflammatory cytokines (IL-17A, IFN $\gamma$ ) but did not influence the response of duodenal memory T cells towards a gliadin peptide (35). A challenge to the hookworm-approach is establishing adequate amounts of applied hookworm larvae as heavy hookworm infections can cause serious adverse effects and underdosing dilutes the desensitization effect (34). Purification, formulation, and storage of the hookworm larvae may also be pharmaceutically challenging and could have an influence on adverse reactions and compliance. Due to both, pharmaceutical challenges and lack of clinical benefit for CD patients, the efficiency of this approach remains debatable.

### Polymeric Binders

Polymeric binders are being evaluated as a means to sequester gluten in the GI tract (36). Poly(hydroxyethyl methacrylate-*co*-styrene sulfonate) (P(HEMA-*co*-SS), BL-7010, BioLineRx) was shown to complex gliadin and decrease the production of immunostimulatory peptides in digestive fluids (37,38). In a mouse model of gluten sensitivity, the polymer diminished the mucosal and systemic response to



**Fig. 1** Many therapeutic strategies are being explored in CD (in blue). Pharmaceutical scientists face interesting challenges in overcoming shortcomings with potentially negative effects on compliance and therapeutic benefit (in red).

an oral gluten-challenge. In addition, the polymer was safe and the systemic exposure was found to be minimal in rodents (38). In *ex vivo* experiments with biopsy specimens from CD patients, secretion of the proinflammatory

cytokine  $\text{TNF}\alpha$  was lowered upon gliadin exposure in the presence of P(HEMA-co-SS) (38). An important advantage of the polymeric binder P(HEMA-co-SS) is its ability to sequester gluten under acidic and neutral conditions (i.e.,

**Table 1** Adjuvant Therapies Under Investigation for CD. The Clinical Trials from this Table are Listed on [clinicaltrials.gov](http://clinicaltrials.gov) (Status as of November 2012)

Therapeutic strategy	Compound	Progress	Reference
Desensitization	Nexvax2®-vaccine	Phase I	(33)
	<i>Necator americanus</i> -inoculation	Phase II	(34)
Oral enzyme supplementation	AN-PEP	Phase II	(43)
	ALV003	Phase II	(45)
Polymeric binders	P(HEMA-co-SS) (BL-7010)	Preclinical	(38)
Modulators of paracellular permeability	Larazotide acetate (AT-1001)	Phase II	(52)
TG2-inhibitors	R281, ZED1098, ZED1219, ZED1227	Preclinical	(56,58)
HLA-blockers	Several compounds	Discovery	(60)

in the stomach and the small intestine) (37). The binding of the polymer to important nutrients has been pointed out as a potential drawback of this approach. However, since the treatment is to be used only occasionally, nutritional deficiencies should not be of concern. The sequestration of enough gluten to achieve a clinical response will remain the most important challenge in the forthcoming clinical trials.

### Oral Enzyme Supplementation

One of the most studied therapeutic options for CD is the oral administration of gluten-degrading enzymes (“glutenases”). Mixtures of germinating wheat proteases and purified prolyl endopeptidases (PEPs) have been reported to hydrolyse proline-rich gluten and to reduce their T cell toxicity *in vitro* (39,40). PEPs from different bacterial (FM, MX, and SC PEP from *Flavobacterium meningosepticum*, *Myxococcus xanthus*, and *Sphingomonas capsulata*, respectively) and fungal species (AN PEP from *Aspergillus niger*) have been extensively studied (41,42). In a pilot clinical trial (NCT00810654), AN PEP (DSM Food Specialties) or placebo-containing jam was administered to CD patients with a breakfast comprising 7 g of gluten twice in 2 weeks. No significant histopathological or serological differences between AN PEP and placebo were observed as too few patients in the control group showed a disease flare (43). AN PEP is particularly active to degrade gluten in the stomach and is being considered as potential food supplement. ALV003 (Alvine Pharmaceuticals) is a mixture of two proteases, i.e. SC PEP and EP-B2. The latter is the self-activating isoform 2 of cysteine endoprotease B from germinated barley seeds, a glutamine-specific endoprotease particularly resistant to low pH and pepsin (44). In a phase I clinical trial (NCT00669825), ALV003 was applied as a powder dissolved in water *via* a nasogastric tube subsequent to a gluten-containing meal (1 g of gluten). Apart from being well tolerated, three hundred mg of ALV003 degraded 80% of administered gluten, which was significantly different from placebo (44). A phase IIa study showed the capacity of nine hundred mg of ALV003 to protect against small-intestinal mucosal injury and to reduce IEL density compared to placebo after a 6 weeks daily gluten challenge (2 g gluten/day) (45). Unfortunately, this trial was underpowered to achieve significant differences in serology or symptoms (45). Other clinical trials are ongoing, and ALV003 was subsequently granted Fast Track designation from the FDA. A major challenge of the glutenase approach is the susceptibility of PEPs to the harsh conditions of the GI tract (46). In rats, the activity of some PEPs was shown to be strongly dependent upon the stomach pH (47) such that food composition will have an influence on therapeutic benefit. To circumvent this problem, MX PEP has been

formulated with enteric capsules (48). However, ideally the degradation of gluten in the stomach should be as complete as possible to suppress an immune response in the proximal small intestine. Furthermore, the time and point of release of PEPs from enteric capsules fluctuates in relation to food amount and content as well as intestinal pH. To increase the stability of gluten-degrading enzymes in the stomach and the small intestine, protein-engineering approaches and covalent modification of PEPs with polymers are being investigated (49,50). These strategies may be viewed as costly but they represent very interesting research challenges for the pharmaceutical scientist as findings stemming from this research could be applied to other GI diseases and oral enzyme therapeutics.

### Modulator of Paracellular Permeability

The enhanced permeability of the intestinal mucosa in active CD is partly caused by gliadin-mediated opening of tight junctions (12). Larazotide acetate (AT-1001, Alba Pharmaceuticals) is a peptidic tight junction regulator that was shown to preserve tight junction structure of the intestinal epithelium both *in vitro* and in gliadin-sensitized mice upon gliadin-administration (51). In a recently reported phase IIa study (NCT00362856), larazotide acetate was applied in capsules before a gluten-containing meal thrice per day for 14 days (2.4 g of gluten/day in total) (52). No statistical significance between larazotide acetate and placebo was achieved for the primary outcome measure for efficacy (lactulose-to-mannitol ratio to assess intestinal permeability) (52). However, symptom severity determined with a questionnaire to quantify GI symptoms was significantly lower for the treatment group after gluten challenge (52). Finding appropriate markers for intestinal permeability is challenging. The reliability of lactulose as a marker for increased paracellular leakage of gliadins was contested with regard to the molecular weight of lactulose (342 Da) compared to immunogenic gliadin peptides such as p31-49 and 33mer (2,245 and 3,900 Da, respectively) (53). A fluorescently-labeled gliadin fragment may be of higher relevance (54). One potential limitation of paracellular blockade is the remaining systemic access of gliadin *via* the transcellular route (IgA-gliadin-conjugates bind the TfR and are transcytosed) (13). Competitive inhibition of gliadin-binding to IgA or IgA-binding to TfR could be achieved with non-immunogenic peptides and proteins but their susceptibility to degradation in the GI tract should be verified. To increase their resistance against proteolysis, the introduction of D-amino acids or cyclization represent interesting avenues to explore.

### TG2-Inhibitors and HLA-Blockers

Inhibition of TG2 abolishes TG2-mediated deamidation of immunogenic gliadin peptides and thus decreases their

avidity to HLA-DQ2 and DQ8. Several reversible and irreversible TG2-inhibitors have been proposed (55). The TG2-inhibitor R281 has been tested *in vitro* and *ex vivo* and showed lowered gliadin-induced toxicity (56). The safety and efficacy of TG2-inhibitors have not been evaluated in humans. Adverse effects due to TG2's ubiquity and sequence-similarities to the active site of other human transglutaminases are possible (15). In TG2-knockout mice, macrophage-mediated phagocytosis of apoptotic cells was disturbed leading to splenomegaly, autoimmunity and immune complex glomerulonephritis (57). Three highly soluble and GI-stable peptidomimetic TG2-inhibitors (ZED1098, ZED1219 and ZED1227, Zedira) with very high selectivity for TG2 were recently developed (58). Their permeation across the GI mucosa will need to be carefully assessed to make sure that their concentration in the *lamina propria* is sufficient. A challenge to TG2-inhibitors is the development of an oral delivery system for maximal GI exposure of the drug. The inhibitor should cross the GI mucosa in sufficiently high concentrations while systemic exposure should be as low as possible. TG2-inhibitors might prove more useful in combination therapy since some gluten peptides are immunogenic without TG2-mediated deamidation (59). Another approach consists in blocking HLA-DQ2 and DQ8 with high avidity peptidic ligands in order to inhibit the presentation of immunogenic gluten-derived peptides by APCs (60). Challenging aspects of the HLA-blocking approach are the peptidic nature of the competitive HLA-blockers, which makes them prone to degradation in the GI tract, the need for high concentration and avidity, as well as possible interference with presentation of peptides from pathogens on HLA-DQ2 and DQ8 (8).

### Anti-Inflammatory Therapy

Modulation of proinflammatory pathways can be achieved with corticosteroids and biologics. Budesonide, a glucocorticoid with low oral bioavailability, was clinically beneficial for refractory (i.e., non-responsive to a GFD) and non-refractory CD (61,62). Its use in CD would require a formulation targeting the proximal small intestine given that budesonide is currently mostly used for inflammatory bowel diseases affecting the ileum and colon (24). Several proinflammatory cytokines and lymphocyte-recruiting chemokines (e.g., TNF $\alpha$ , IFN $\gamma$ , IL-15, CCL25, CXCL10) were recently identified to be particularly relevant in CD (31). For some of these targets, biological drugs are either being developed or are already on the market but for other indications. For example, a human IgG1 anti-IL-15 monoclonal antibody (AMG-714, Amgen) was evaluated in patients with rheumatoid arthritis (63), but was discontinued for this indication due to a disappointing phase II study (64). Infliximab (Remicade®, Janssen Biotech), a monoclonal antibody blocking TNF $\alpha$  used in RA and Crohn's disease, had positive effects on a patient with refractory CD after failure of corticosteroid therapy (65). However, clinical trials with

outcome measures designed for CD do not exist for most anti-inflammatory agents. Severe adverse effects and high costs (66) may disqualify TNF $\alpha$  blockers for non-refractory CD, as a GFD is a relatively safe and inexpensive alternative (8). Moreover, periodic administration of biological drugs by trained personnel is expensive and might be addressed by subcutaneous self-injection devices or by developing compounds with long circulation times (e.g., PEGylation technologies) (67).

### CONCLUSION

CD is an immune-mediated enteropathy affecting 1% of the population and rising in prevalence. Because of growing awareness of gluten-related disorders, avoidance of dietary gluten is increasingly common, even in non-celiacs. With no pharmacologic treatments currently available, formulation scientists may have a pivotal role to play in the development of clinically viable pharmacological options. Specific delivery systems are required to reduce adverse effects and to transport drugs to their site of action. Improvements in the formulations of peptide- and protein-based drugs are needed to enhance their stability and activity, and will therefore be highly instrumental in ameliorating treatment strategies for CD. Pharmaceutical scientists can make significant contributions to reduce the burden on CD patients and make a significant impact on their quality of life.

### ACKNOWLEDGMENTS AND DISCLOSURES

Financial support from the Swiss National Science Foundation (310030\_135732) and "IG Zöliakie der Deutschen Schweiz" is acknowledged. Dr. Marc A. Gauthier is acknowledged for his critical reading of the manuscript.

J.-C. Leroux has a consultancy agreement with BioLineRx. The remaining authors disclose no conflicts of interest.

### REFERENCES

1. Ludvigsson JF, Leffler DA, Bai JC, Biagi F, Fasano A, Green PHR, *et al.* The Oslo definitions for coeliac disease and related terms. *Gut*. 2012. doi:10.1136/gutjnl-2011-301346.
2. Tack GJ, Verbeek WHM, Schreurs MWJ, Mulder CJJ. The spectrum of celiac disease: epidemiology, clinical aspects and treatment. *Nat Rev Gastroenterol Hepatol*. 2010;7(4):204–13.
3. Sollid LM. Coeliac disease: dissecting a complex inflammatory disorder. *Nat Rev Immunol*. 2002;2(9):647–55.
4. Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. The prevalence of celiac disease in the United States. *Am J Gastroenterol*. 2012;107(10):1538–44.

5. Catassi C, Kryszak D, Bhatti B, Sturgeon C, Helzlsouer K, Clipp SL, *et al.* Natural history of celiac disease autoimmunity in a USA cohort followed since 1974. *Ann Med.* 2010;42(7):530–8.
6. Rewers M, Eisenbarth GS. Autoimmunity: celiac disease in T1DM—the need to look long term. *Nat Rev Endocrinol.* 2012;8(1):7–8.
7. Di Sabatino A, Corazza GR. Coeliac disease. *Lancet.* 2009;373(9673):1480–93.
8. Pinier M, Fuhrmann G, Verdu E, Leroux J-C. Prevention measures and exploratory pharmacological treatments of celiac disease. *Am J Gastroenterol.* 2010;105(12):2551–61.
9. Ciccocioppo R, Di Sabatino A, Corazza GR. The immune recognition of gluten in coeliac disease. *Clin Exp Immunol.* 2005;140(3):408–16.
10. Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM, *et al.* Structural basis for gluten intolerance in celiac sprue. *Science.* 2002;297(5590):2275–9.
11. Heyman M, Abed J, Lebreton C, Cerf-Bensussan N. Intestinal permeability in coeliac disease: insight into mechanisms and relevance to pathogenesis. *Gut.* 2012;61(9):1355–64.
12. Fasano A. Leaky gut and autoimmune diseases. *Clin Rev Allergy Immunol.* 2012;42(1):71–8.
13. Lebreton C, Ménard S, Abed J, Moura IC, Coppo R, Dugave C, *et al.* Interactions among secretory immunoglobulin A, CD71, and transglutaminase-2 affect permeability of intestinal epithelial cells to gliadin peptides. *Gastroenterology.* 2012;143(3):698–707.
14. Molberg O, McAdam S, Lundin KEA, Kristiansen C, Arentz-Hansen H, Kett K, *et al.* T cells from celiac disease lesions recognize gliadin epitopes deamidated *in situ* by endogenous tissue transglutaminase. *Eur J Immunol.* 2001;31(5):1317–23.
15. Di Sabatino A, Vanoli A, Giuffrida P, Luinetti O, Solcia E, Corazza GR. The function of tissue transglutaminase in celiac disease. *Autoimmun Rev.* 2012;11(10):746–53.
16. Meresse B, Malamut G, Cerf-Bensussan N. Celiac disease: an immunological jigsaw. *Immunity.* 2012;36(6):907–19.
17. Maiuri L, Ciacci C, Ricciardelli I, Vacca L, Raia V, Auricchio S, *et al.* Association between innate response to gliadin and activation of pathogenic T cells in coeliac disease. *Lancet.* 2003;362(9377):30–7.
18. Abadie V, Discepolo V, Jabri B. Intraepithelial lymphocytes in celiac disease immunopathology. *Semin Immunopathol.* 2012;34(4):551–66.
19. DePaolo RW, Abadie V, Tang F, Fehlner-Peach H, Hall JA, Wang W, *et al.* Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. *Nature.* 2011;471(7337):220–4.
20. Sapone A, Bai J, Ciacci C, Dolinsek J, Green P, Hadjivassiliou M, *et al.* Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Med.* 2012;10(1):13.
21. Fasano A. Clinical presentation of celiac disease in the pediatric population. *Gastroenterology.* 2005;128(4):S68–73.
22. Sapone A, Lammers K, Casolaro V, Cammarota M, Giuliano M, De Rosa M, *et al.* Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. *BMC Med.* 2011;9(1):23.
23. Ahn R, Ding YC, Murray J, Fasano A, Green PHR, Neuhausen SL, *et al.* Association analysis of the extended MHC region in celiac disease implicates multiple independent susceptibility loci. *PLoS One.* 2012;7(5):e36926.
24. Sollid LM, Khosla C. Novel therapies for coeliac disease. *J Intern Med.* 2011;269(6):604–13.
25. Trynka G, Wijmenga C, van Heel DA. A genetic perspective on coeliac disease. *Trends Mol Med.* 2010;16(11):537–50.
26. Lindfors K, Koskinen O, Kaukinen K. An update on the diagnostics of celiac disease. *Int Rev Immunol.* 2011;30(4):185–96.
27. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, *et al.* European society for pediatric gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease. *J Pediatr Gastroenterol Nutr.* 2012;54(1):136–60.
28. Barratt SM, Leeds JS, Sanders DS. Quality of life in coeliac disease is determined by perceived degree of difficulty adhering to a gluten-free diet, not the level of dietary adherence ultimately achieved. *J Gastrointest Liver Dis.* 2011;20(3):241–5.
29. Tio M, Cox MR, Eslick GD. Meta-analysis: coeliac disease and the risk of all-cause mortality, any malignancy and lymphoid malignancy. *Aliment Pharmacol Ther.* 2012;35(5):540–51.
30. Stoven S, Murray JA, Marietta E. Celiac disease: advances in treatment *via* gluten modification. *Clin Gastroenterol Hepatol.* 2012;10(8):859–62.
31. Rashtak S, Murray JA. Review article: coeliac disease, new approaches to therapy. *Aliment Pharmacol Ther.* 2012;35(7):768–81.
32. Keech CL, Dromey J, Chen Z, Anderson RP, McCluskey J. Immune tolerance induced by peptide immunotherapy in an HLA Dq2-dependent mouse model of gluten immunity. *Gastroenterology.* 2009;136(5):A-57.
33. Brown GJ, Daveson J, Marjason JK, Ffrench RA, Smith D, Sullivan M, *et al.* A phase I study to determine safety, tolerability and bioactivity of Nexvax2® in HLA DQ2+ volunteers with celiac disease following a long-term, strict gluten-free diet. *Gastroenterology.* 2011;140(5):S-437–8.
34. Daveson AJ, Jones DM, Gaze S, McSorley H, Clouston A, Pascoe A, *et al.* Effect of hookworm infection on wheat challenge in celiac disease—a randomised double-blinded placebo controlled trial. *PLoS One.* 2011;6(3):e17366.
35. McSorley HJ, Gaze S, Daveson J, Jones D, Anderson RP, Clouston A, *et al.* Suppression of inflammatory immune responses in celiac disease by experimental hookworm infection. *PLoS One.* 2011;6(9):e24092.
36. Bertrand N, Gauthier MA, Bouvet C, Moreau P, Petitjean A, Leroux J-C, *et al.* New pharmaceutical applications for macromolecular binders. *J Control Release.* 2011;155(2):200–10.
37. Pinier M, Verdu EF, Nasser-Eddine M, David CS, Vézina A, Rivard N, *et al.* Polymeric binders suppress gliadin-induced toxicity in the intestinal epithelium. *Gastroenterology.* 2009;136(1):288–98.
38. Pinier M, Fuhrmann G, Galipeau HJ, Rivard N, Murray JA, David CS, *et al.* The copolymer P(HEMA-co-SS) binds gluten and reduces immune response in gluten-sensitized mice and human tissues. *Gastroenterology.* 2012;142(2):316–25.
39. Marti T, Molberg O, Li Q, Gray GM, Khosla C, Sollid LM. Prolyl endopeptidase-mediated destruction of T cell epitopes in whole gluten: chemical and immunological characterization. *J Pharmacol Exp Ther.* 2005;312(1):19–26.
40. Stenman SM, Venäläinen JI, Lindfors K, Auriola S, Mauriala T, Kaukovirta-Norja A, *et al.* Enzymatic detoxification of gluten by germinating wheat proteases: implications for new treatment of celiac disease. *Ann Med.* 2009;41(5):390–400.
41. Fuhrmann G, Leroux J-C. *In vitro* evaluation of the stability of proline-specific endopeptidases under simulated gastrointestinal conditions. *J Control Release.* 2010;148(1):e37–9.
42. Stepniak D, Spaenij-Dekking L, Mitea C, Moester M, de Ru A, Baak-Pablo R, *et al.* Highly efficient gluten degradation with a newly identified prolyl endoprotease: implications for celiac disease. *Am J Physiol Gastrointest Liver Physiol.* 2006;291(4):G621–9.
43. Tack GJ, van de Water JM, Kooy-Winkelaar EM, van Bergen J, Meijer GA, von Blomberg BM, *et al.* Can prolyl endoprotease enzyme treatment mitigate the toxic effect of gluten in coeliac patients? *Gastroenterology.* 2010;138(5):S-54.
44. Siegel M, Garber ME, Spencer AG, Botwick W, Kumar P, Williams RN, *et al.* Safety, tolerability, and activity of ALV003: results from two phase I single, escalating-dose clinical trials. *Dig Dis Sci.* 2012;57(2):440–50.



45. Adelman DC. Gluten degradation by ALV003, a novel drug in development for coeliac disease. 26th AOECS general assembly, international coeliac disease scientific conference—better life for coeliacs. 2012. 6.-9.09.2012, Helsinki, Finland (2012).
46. Shan L, Marti T, Sollid LM, Gray GM, Khosla C. Comparative biochemical analysis of three bacterial prolyl endopeptidases: implications for coeliac sprue. *Biochem J*. 2004;383(2):311–8.
47. Fuhrmann G, Leroux J-C. *In vivo* fluorescence imaging of exogenous enzyme activity in the gastrointestinal tract. *Proc Natl Acad Sci U S A*. 2011;108(22):9032–7.
48. Gass J, Ehren J, Strohmeier G, Isaacs I, Khosla C. Fermentation, purification, formulation, and pharmacological evaluation of a prolyl endopeptidase from *Myxococcus xanthus*: Implications for Celiac Sprue therapy. *Biotechnol Bioeng*. 2005;92(6):674–84.
49. Ehren J, Govindarajan S, Morón B, Minshull J, Khosla C. Protein engineering of improved prolyl endopeptidases for coeliac sprue therapy. *Protein Eng Des Sel*. 2008;21(12):699–707.
50. Robic S. Alvine Pharmaceuticals Inc, USA, assignee. PEGylated Glutenase Polypeptides. Patent WO2007047303A2. 2007.
51. Gopalakrishnan S, Durai M, Kitchens K, Tamiz AP, Somerville R, Ginski M, *et al*. Larazotide acetate regulates epithelial tight junctions *in vitro* and *in vivo*. *Peptides*. 2012;35(1):86–94.
52. Leffler DA, Kelly CP, Abdallah HZ, Colatrella AM, Harris LA, Leon F, *et al*. A randomized, double-blind study of larazotide acetate to prevent the activation of coeliac disease during gluten challenge. *Am J Gastroenterol*. 2012;107(10):1554–62.
53. Ménard S, Lebreton C, Schumann M, Matysiak-Budnik T, Dugave C, Bouhnik Y, *et al*. Paracellular *versus* transcellular intestinal permeability to gliadin peptides in active coeliac disease. *Am J Pathol*. 2012;180(2):608–15.
54. Mazumdar K, Alvarez X, Borda JT, Dufour J, Martin E, Bethune MT, *et al*. Visualization of transepithelial passage of the immunogenic 33-residue peptide from  $\alpha$ -2 gliadin in gluten-sensitive macaques. *PLoS One*. 2010;5(4):e10228.
55. Siegel M, Khosla C. Transglutaminase 2 inhibitors and their therapeutic role in disease states. *Pharmacol Ther*. 2007;115(2):232–45.
56. Rauhavirta T, Oittinen M, Kivistö R, Männistö P, Garcia-Horsman J, Wang Z, *et al*. Are transglutaminase 2 inhibitors able to reduce gliadin-induced toxicity related to coeliac disease? A proof-of-concept study. *J Clin Immunol*. doi:10.1007/s10875-012-9745-5.
57. Szondy Z, Sarang Z, Molnár P, Németh T, Piacentini M, Mastroberardino PG, *et al*. Transglutaminase 2<sup>-/-</sup> mice reveal a phagocytosis-associated crosstalk between macrophages and apoptotic cells. *Proc Natl Acad Sci U S A*. 2003;100(13):7812–7.
58. Hils M, Weber J, Buechold C, Pasternack R. Selective blockers of tissue transglutaminase for coeliac disease therapy. 26th AOECS general assembly, international coeliac disease scientific conference—better life for coeliacs. 2012. 6.-9.09.2012, Helsinki, Finland (2012).
59. van de Wal Y, Kooy YMC, van Veelen P, Vader W, August SA, Drijfhout JW, *et al*. Glutenin is involved in the gluten-driven mucosal T cell response. *Eur J Immunol*. 1999;29(10):3133–9.
60. Kapoerchan VV, Wiesner M, Overhand M, van der Marel GA, Koning F, Overkleeft HS. Design of azidoproline containing gluten peptides to suppress CD4<sup>+</sup> T-cell responses associated with coeliac disease. *Bioorg Med Chem*. 2008;16(4):2053–62.
61. Brar P, Lee S, Lewis S, Egbuna I, Bhagat G, Green PHR. Budesonide in the treatment of refractory coeliac disease. *Am J Gastroenterol*. 2007;102(10):2265–9.
62. Daum S, Ipczynski R, Heine B, Schulzke JD, Zeitz M, Ullrich R. Therapy with budesonide in patients with refractory sprue. *Digestion*. 2006;73(1):60–8.
63. Baslund B, Tvede N, Danneskiold-Samsøe B, Larsson P, Panayi G, Petersen J, *et al*. Targeting interleukin-15 in patients with rheumatoid arthritis: a proof-of-concept study. *Arthritis Rheum*. 2005;52(9):2686–92.
64. Šenolt L, Vencovský J, Pavelka K, Ospelt C, Gay S. Prospective new biological therapies for rheumatoid arthritis. *Autoimmun Rev*. 2009;9(2):102–7.
65. Costantino G, della Torre A, Lo Presti MA, Caruso R, Mazzon E, Fries W. Treatment of life-threatening type I refractory coeliac disease with long-term infliximab. *Dig Liver Dis*. 2008;40(1):74–7.
66. Aaltonen KJ, Virkki LM, Malmivaara A, Kontinen YT, Nordström DC, Blom M. Systematic review and meta-analysis of the efficacy and safety of existing TNF blocking agents in treatment of rheumatoid arthritis. *PLoS One*. 2012;7(1):e30275.
67. Gauthier MA, Klok H-A. Polymer-protein conjugates: an enzymatic activity perspective. *Polym Chem*. 2010;1(9):1352–73.