Development of drugs for celiac disease: review of endpoints for Phase 2 and 3 trials

Klaus Gottlieb1,*, Jill Dawson2, Fez Hussain1 and Joseph A. Murray3

1Immunology and Internal Medicine - Medical Strategy & Science, Quintiles, Durham, NC, USA, 2Corporate Communications, Quintiles, Durham, NC, USA and 3Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, MN, USA

*Corresponding author. Immunology and Internal Medicine - Medical Strategy & Science, Quintiles, 4820 Emperor Boulevard, Durham, NC 27703, USA. Tel: +1-866-267-4479; Email: klaus.gottlieb@quintiles.com

Abstract

Celiac disease is a lifelong disorder for which there is currently only one known, effective treatment: a gluten-free diet. New treatment approaches have recently emerged; several drugs are in Phase 2 trials and results appear promising; however, discussion around regulatory endpoints is in its infancy. We will briefly discuss the drugs that are under development and then shift our attention to potential trial endpoints, such as patient-reported outcomes, histology, serology, gene expression analysis and other tests. We will outline the differing requirements for proof-of-concept Phase 2 trials and Phase 3 registration trials, with a particular emphasis on current thinking in regulatory agencies. We conclude our paper with recommendations and a glossary of regulatory terms, to enable readers who are less familiar with regulatory language to take maximum advantage of this review.

Key words: celiac disease; clinical trials; endpoints

Introduction

Research into therapy for celiac disease is currently at a particularly interesting point. There are now several different drugs in development but no agreement exists on the best endpoints for registration trials. We will review drugs in the ‘pipeline’, examine the experience to date, and discuss possible regulatory endpoints in detail.

Pathology and incidence of celiac disease

Celiac disease is an immune-mediated small intestinal enteropathy, triggered by the ingestion of gluten in the genetically susceptible, which results in villous atrophy. The presenting symptoms may range from diarrhea, constipation, vomiting, malnutrition, or failure to thrive, to chronic fatigue, joint pain, anemia, osteoporosis, or migraines [1]. The prevalence of celiac disease has increased over the past 50 years and the rate of diagnosis has risen over the past two decades [2].

Celiac disease affects some two million Americans, of whom around 83% are not diagnosed [3, 4], and 3.5 million Europeans [5]. This makes celiac disease one of the most common food-related, lifelong disorders worldwide [6]. Although many individuals with this disease remain undiagnosed, others who have been given the diagnosis do not actually have the disease; additionally, there is an increasing number of people who have no diagnosis of celiac disease but who nevertheless adhere to a gluten-free diet (GFD). Rubio-Tapia et al. reported that, among the general population of the USA that has not been diagnosed with celiac disease, the prevalence of gluten exclusion was...
similar to that of actual celiac disease [7]. The environmental trigger (gluten derived from wheat, rye and barley), the genetic predisposition conferred by the human leukocyte antigen (HLA)-DQ2 and HLA-DQ8 haplotypes, and many steps in the disease pathogenesis are known. Novel alternative treatments or adjunctive therapies to a gluten-free diet—which is currently the only available and effective treatment for the condition—are increasingly being suggested [8].

This paper covers celiac disease per se, which is the best-characterized of the spectrum of gluten-related disorders [9]. A gluten-free diet is central to the management of celiac disease and has historically been the only treatment, although several potential therapies are now under development. There is also a widespread public interest in gluten-free foods reflected, for example, in recent U.S. Food and Drug Administration (the FDA) food labeling regulations [10]. Market research by the National Purchase Diary Group (NPD) shows a gradual but steady increase in the percentage of adults who say they are cutting down on gluten or avoiding it completely—currently more than one in every four adults [11].

Celiac disease is typically detected by serological testing of celiac-specific antibodies, and the diagnosis is usually confirmed by duodenal mucosal biopsies [12]. Both serology and biopsy should be performed while the individual is following a gluten-containing diet [12]; however, potential celiac disease sufferers who have started themselves on a celiac-free diet before a work-up was completed, may be harder to diagnose. This is especially true if the patient carries the HLA-DQ2 and HLA-DQ8 haplotypes, as do 30-50% of the unaffected population [13]. In these circumstances, a new diagnostic test—currently still under development and based on cytokine release assay in response to a gluten challenge—could be helpful [14]. Although duodenal biopsies are still considered essential in adults, an alternative diagnostic strategy has been suggested, which avoids biopsies in children who have tissue transglutaminase levels higher than 10 times the upper limit of normal [15].

It is generally believed that the IgA anti-tissue transglutaminase (tTG) is the single best serological test to use for the detection of celiac disease. While celiac disease can be recognized endoscopically by visual inspection, especially if water immersion is used to enhance the detection of villi, a normal endoscopic appearance does not preclude the diagnosis [16].

At present, treatment primarily involves a GFD, an approach that demands significant patient education, motivation, and follow-up. The GFD also imposes a heavy treatment burden on patients, given the requirement for constant vigilance for gluten contamination. Not surprisingly, a large proportion of patients report inadvertent or deliberate exposure to gluten [17]. Non-responsive celiac disease is frequently reported, especially among individuals diagnosed in adulthood, and may be in part related to trace gluten contamination [18]. In many adult patients—perhaps the majority—the intestine fails to heal even after two years of the GFD [19].

Research and development ‘pipeline’ for celiac disease drugs & continuing clinical trials

Clinical trials on drugs to treat celiac disease are at an early stage of evolution, with no products having reached Phase 3 to date (Figure 1). Products in development include:

- ALV003, Alvine Pharmaceuticals’ lead clinical candidate for celiac disease, which is currently being studied in Phase 2b [20]. ALV003 is an orally administered mixture of two recombinant gluten-specific proteases—a cysteine protease (EP-B2) and a prolyl endopeptidase (PEP)—which have been shown in vitro to degrade gluten.

In a Phase 2 study with ALV003, adults with biopsy-proven celiac disease were randomly assigned to groups receiving ALV003 (n = 20) or placebo (n = 21), together with a daily 2 g gluten challenge. Duodenal biopsies were collected at baseline and after the gluten challenge. The ratio of villus-height-to-crypt-depth and densities of intra-epithelial lymphocytes were the primary endpoints. Biopsies from subjects in the placebo group showed evidence of mucosal injury after gluten challenge (mean villus height-to-crypt depth ratio changed from 2.8 before challenge to 2.0 afterward; P = 0.0007; density of CD3+ intraepithelial lymphocytes changed from 61 to 91 cells/mm^2 after challenge; P = 0.0003). In contrast, no significant mucosal deterioration was observed in biopsies from the ALV003 group. Between groups, morphologic changes and CD3+ intraepithelial lymphocyte counts differed significantly from baseline to Week 6 (P = 0.0133 and P = 0.0123, respectively). Interestingly, there were no statistically significant differences in symptoms between groups [21].

Based on the promising Phase 2a results, a Phase 2b study is now being conducted. The study is evaluating the safety and efficacy of ALV003 at different dose levels administered over a twelve-week period in 500 celiac disease patients in the USA, Canada and Europe, who are symptomatic despite attempting to follow a GFD. The primary efficacy endpoint for the study is the change in small intestinal mucosal morphology, as measured by the change in villus-height-to-crypt-depth ratio (Vh:Cd) from baseline and week 12 assessments. Secondary endpoints are the changes in density in intestinal intraepithelial lymphocytes and celiac disease-specific symptoms during the study. Other outcomes to be evaluated include changes in celiac disease serologies and quality-of-life measures [20].

Aspergillus niger prolyl endopeptidase (AN-PEP) is an endopeptidase, like the PEP component of ALV003, which can break down gluten. The enzyme is active between pH 2 and pH 8, with an optimum activity at pH 4–5, and is therefore effective at the pH levels present in the stomach and small intestine [22]. AN-PEP was evaluated in a recent small, double-blind, placebo-controlled, randomized trial on 16 patients with a diagnosis of celiac disease—as confirmed by positive serology—with sub-total or total villous atrophy on duodenal biopsies, who adhered to a strict GFD, resulting in normalized antibodies and mucosal healing classified as Marsh 0 or I [22]. In a randomized, double-blind, placebo-controlled pilot study, patients consumed toast (approximately 7 g/day gluten) with AN-PEP for 2 weeks (safety phase). After a 2-week washout period with adherence to the usual GFD, 14 patients were randomized to gluten intake with either AN-PEP or placebo for 2 weeks (efficacy phase). No serious adverse events occurred and no patients withdrew during the trial. The mean score for the gastrointestinal subcategory of the celiac disease quality (CDQ) was relatively high throughout the study, indicating that AN-PEP was well tolerated. In the efficacy phase, the CDQ scores of patients consuming gluten with placebo or gluten with AN-PEP did not significantly deteriorate and, moreover, no differences between the groups were observed. The authors conclude in their discussion that “with hindsight, the study should possibly have been designed for a much longer period of time with many more patients” [22].

- Larazotide acetate (AT-1001) is Alba Therapeutics Corporation’s investigational product, a first-in-class tight junction regulator,
intended for the treatment of patients with celiac disease. It has been hypothesized that celiac disease is accompanied by raised paracellular permeability, accompanied by an inflammatory cascade within the bowel, which is controlled by tight junctions. Alba has reported positive results from a double-blind, placebo-controlled, Phase 2b trial in February 2014 [23]. This evaluated the efficacy and safety of larazotide acetate in 342 celiac disease patients who had symptoms despite being on a GFD. The trial consisted of a four-week placebo run-in, 12 weeks of randomized therapy, and four weeks of post-treatment follow-up. Patients were randomized to four groups: a placebo group or larazotide 0.5, 1.0, or 2.0 mg, three times per day. Treatment with the lowest of three doses of larazotide was associated with significant improvement in the primary outcome, i.e. the average on-treatment score in the Celiac Disease Gastrointestinal Symptom Rating Scale (CeD GSRS) domains of Diarrhea, Indigestion, and Abdominal pain. Analysis of individual components of the rating scale, proprietary to Alba Therapeutics [24], showed consistent improvement with larazotide for each parameter [25].

The Phase 2b study discussed above was preceded by a separate, dose-ranging, placebo-controlled study of 86 patients with celiac disease controlled through diet [26]. The aim of this study was to evaluate the efficacy and tolerability of larazotide in protecting against gluten-induced intestinal permeability and worsening gastrointestinal symptoms. Study participants were randomly assigned to larazotide acetate (0.25, 1, 4, or 8 mg) or placebo three times a day, with or without gluten challenge (2.4 g/day) for 14 days. The primary efficacy outcome, an improvement in the lactulose/mannitol (LAMA) fractional excretion ratio (an experimental biomarker for intestinal permeability) was not met; however, the 0.25 and 4.0 mg doses of larazotide acetate showed statistically significant prevention of severe worsening of gastrointestinal symptoms.

An exploratory study published in 2013 [27] examined the effect of larazotide acetate on intestinal permeability, development of antibodies to tTG and celiac disease symptoms during a gluten challenge that exceeded the likely level of accidental gluten ingestion in individuals whose disease was well controlled by a GFD. In the larazotide acetate 1 mg group, a reduction in the expected increase was seen in the urinary LAMA ratio but the difference was not statistically significant as compared with placebo. Changes in pre-specified secondary endpoints suggest that larazotide acetate reduced antigen exposure, as shown by lowered production of anti-tTG antibodies. Larazotide acetate also reduced gastrointestinal symptoms upon gluten challenge.

- Nexvax2: ImmusanT’s peptide-based therapeutic celiac disease vaccine.

According to press releases from ImmusanT [28], the therapeutic vaccine Nexvax2 combines three proprietary peptides that elicit an immune response in celiac disease patients who carry the immune recognition gene HLA-DQ2. Similarly to treatments for allergies, the vaccine is designed to reprogram gluten-specific T cells triggered by the patient’s immune response to the protein. According to ImmusanT, the objective is for Nexvax2 to restore celiac patients’ immune tolerance to gluten, reduce inflammation in the nutrient-absorbing villi that line the small intestine, return the intestine to a healthy state, and allow patients to eat a normal diet [28].

The company says that early clinical trials have so far proven promising, with Phase 1b trial results demonstrating clear proof of mechanism and Phase 2 trials expected to begin in 2015—but details are not known at the time of this writing (December 2014) [29].

- BL-7010: BiolineRx’s non-absorbable, high molecular weight polymer with a high affinity for gliadins, the immunogenic peptides present in gluten that cause celiac disease. The product acts by sequestering gliadins. Experiments in vivo in a murine model of celiac disease have shown that BL-7010 prevents pathological damage to the small intestine, helps to preserve the integrity of
the intestinal mucosa and reduces inflammation [36]. Although the company website lists BL-7010 as being in pre-clinical development, clinicaltrials.gov shows an active recruiting Phase 1 safety study [31].

- AVX176, from Avaxia Biologics, is an investigational oral antibody drug that is the subject of U.S. composition of matter patent 8,071,101, "Antibody Therapy for Treatment of Diseases Associated with Gluten Intolerance." The patent, which expires on May 27 2029, provides broad coverage for treating celiac disease using orally administered antibodies produced by Avaxia’s proprietary platform technology [32].

- ActoGenX is carrying out discovery research in celiac disease with its range of ActoBiotics™, which use Lactococcus lactis as an expression system to locally secrete bio-therapeutics such as cytokines, antibodies, hormones, etc. [33]. Early pre-clinical work with a genetically altered L. lactis secreting a peptide derived from gliadin demonstrated an in vivo suppression of gluten sensitization. Specifically, Huijbregts et al. engineered L. lactis to secrete a deaminated DQ8 gliadin epitope (LL-eDQ8d) and studied the induction of Ag-specific tolerance in NOD Albo DQ8 transgenic mice [34]. Although apparently not part of the ActoGenX development program, recent work by Galipeau et al. also deserves mention in this context: The group treated gluten-sensitive mice with eflafin, a serine protease inhibitor, delivered by the L. lactis vector, and found normalization of inflammation, improved permeability, and maintained ZO-1 expression. There is speculation that this is due to reduced deamidation of gliadin peptide [35].

- Chemocentryx’s CCR9 (vericomin, which is also known as Traficet-EN, or CXCR2828)—originally intended for patients with moderate-to-severe Crohn’s disease—has completed one Phase 2 trial in 67 patients with celiac disease [35]; however, despite the completion of the trial several years ago, no results relating to celiac disease have been made public or published.

A patent search (see Appendix Table A1) revealed at that at least a dozen patents (including U.S. and European patents) were granted for potential celiac disease-related therapies and diagnostics in 2013 and 2014. These were assigned to organizations including Alvine Pharmaceuticals Inc., Curemark LLC, Medarex Inc., Aesku Diagnostics GmbH & Co. KG, Immcro Diagnostics Inc., Alba Therapeutics Corp., Nestec SA, BTG International Ltd., ImmusanT Inc., DSM IP Assets BV, Sigma Tau Ind Farmaceuti, and various institutions, groups and individuals.

### Clinical trial endpoints

An endpoint is quite simply a measure believed to quantify the potential effect of the treatment or intervention under study. Effect is, of course, a term that can be broadly interpreted. The effect is articulated in the claims made on the official drug label and such claims need to be supported by the study results, which are quantified by the endpoints. The process of thinking in terms of a drug’s mechanism of action (what it could do in theory), what it does in practice (clinical effect and efficacy), how this is demonstrated (endpoints) and how this relates into drug labeling claims (what is on the label) is not linear; in fact, regulatory consultants often advise that the thinking should begin around appropriate endpoints, with the end (labeling claims) in mind. In fact, this approach has found its canonization in what is known as the target product profile (TPP). Although not much used in a formal way, it nevertheless reflects how regulators think: the TPP is organized according to the key sections in the drug label and links drug development activities to specific concepts intended for inclusion in the drug labeling [36].

Clinical trial endpoints are well established in many major disease areas where a fair number of registration trials have already been conducted and drugs approved for marketing. Emerging fields, such as celiac disease—where there is little experience and no approved products—often lack agreed endpoints. Consequently, both sponsors and regulators will need to come to a new agreement for each development program. Many small biotechnology companies lack in-house regulatory experience and much frustration could be avoided by understanding, at an early stage, some general principles concerning primary endpoints for registration trials. In the following, we will review important concepts.

For registration trials, the FDA requires ‘clinically meaningful endpoints’ defined as endpoints that are direct measures of how patients feel, function, and survive [37] unless a validated surrogate biomarker acceptable to the FDA is available, which is rare. In disease in which there is a large subjective component, co-primary endpoints are increasingly employed: typically, a patient-reported outcome (PRO) is combined with an instrument that reflects disease activity more directly—for example, a biomarker. This approach is currently being implemented for Crohn’s disease and ulcerative colitis. Although the inflammatory bowel diseases have little in common with celiac disease, they are still similar with regard to ‘treatment success’ which would be incompletely captured if one were to focus on a single endpoint; for example histology or how the patient feels, but not both.

In celiac disease, conceptually, one type of treatment could control symptoms and prevent worsening of damage while another is, at least initially, focused primarily on healing and maintenance of healing, with little effect on symptoms. Obviously, in both cases, different endpoints or endpoint instruments are needed.

Several other food- and allergy-related disorders share a lack of well-defined clinical endpoints. An example is eosinophilic esophagitis, which occurs in response to an as-yet unknown allergen in the diet, has histological manifestations, has histology that does not correlate well with symptoms, and in which there is a requirement for an endpoint instrument that combines a PRO with objective response criteria [38].

Co-primary endpoints require that, to be an overall responder, patients meet the responder definitions for each of the individual endpoints that comprise the co-primary endpoint (see Glossary). Composite endpoints, in contrast, are endpoints that are composed of different measurements (e.g. several different scales, instruments or components) that are aggregated to an overall endpoint (see Glossary).

### Potential celiac disease endpoints

Categories of endpoints for consideration include the following (see Glossary for detailed definitions):

#### Patient-reported outcomes

In some diseases, PROs are more important than in others; compare, for example, depression with hypertension. A PRO is needed as either a primary endpoint or a component of a primary endpoint if the PRO alone is not sufficient to characterize improvements in how the patient feels, functions and survives.

We are aware of three published celiac-specific PRO questionnaires: Dorn 2010 (25 citations) [39], Leffler 2009 (18 citations) [40], and Häuser 2007 (36 citations) [41]. Given its recent publication, the CD-QoL by Dorn et al. has probably attracted the most attention. More generic questionnaires, such as the Gastrointestinal Symptoms Rating Scale (GSRS), have also been...
applied to measure celiac disease-related symptoms. Earlier work criticized the fact that, in celiac disease, the full dynamic range of the GSRS was not used [42]. Others have more recently demonstrated that the GSRS correlates with the ratio of the villus-height-to-crypt-depth—a promising histological outcome measurement instrument—as well as with laboratory test results, numbers of intraepithelial CD3+ cells, and serum levels of antibodies associated with celiac disease [43]. Regulatory authorities seem to have greater familiarity with the GSRS instrument than with others.

Celiac PROs are often described as having been validated, however, it is important to note that the FDA considers a ‘drug development tool’ (DDT) such as a PRO only ‘validated’ when it has undergone the FDA’s DDT qualification process [44]. There is currently only one instrument, the Exacerbations of Chronic Pulmonary Disease Tool for chronic obstructive pulmonary disease, which is validated through this relatively new procedure. The acceptability of any other PRO would have to be decided between the FDA and sponsor on a case-by-case basis.

A proprietary questionnaire being used by Alba in their Phase 2b multi-center trial ‘evaluating the efficacy, safety and tolerability of larazotide acetate in patients with celiac disease’ (see above) is of interest. Alba states that the questionnaire was developed according to the FDA’s Study Endpoints and Labeling Development (SEALD) guidelines, which is the FDA office involved in DDT qualification, and uses PROs [24].

A similar proprietary PRO, called the Celiac Disease Symptom Diary (CDSD), was developed by Alvine and published in abstract form in 2012 [45]. It collects data on symptoms common to celiac disease: abdominal pain, bloating, constipation, diarrhea, fatigue, flatulence, headache, nausea, skin rash, and problems with thinking clearly.

### Histology

Histology instruments used to be considered the ‘gold standard’ for the diagnosis of celiac disease and, indeed, part of the definition of this disease; however, this has been challenged, and the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) guidelines now consider HLA type and the results of serology to be of equal importance [15, 46]. The ESPGHAN criteria allow for the avoidance of biopsy in symptomatic children who have tTG IgA antibodies that are >10 times the upper limit of normal on an initial blood sample, followed by a confirmatory anti-endomysial antibodies (EMA) and HLA typing on a second blood draw. The response to a GFD can also be used to support the diagnosis. The approach outlined in the ESPGHAN guidelines has, however, not been endorsed by other societies with a predominantly adult focus such as the British Gastroenterological Society (BGS) or the American College of Gastroenterology (ACG). However, this approach has not been endorsed by adult societies, such as the British Gastroenterological Society (BGS) or the American College of Gastroenterology (ACG).

Although reasonable for diagnosis (in conjunction with other biomarkers), histology instruments are slow to respond to gluten-free diet, and complete normalization may take years. Another concern, currently unresolved, is the sometimes patchy distribution of disease activity, which can lead to sampling error. Also, there are various systems: Marsh, Marsh

| Publication | Focus | Scoring system |
|-------------|-------|----------------|
| Marsh and Crowe, 1995 [47], Marsh, 1992 [48] | Small intestinal mucosal immunopathology | Concludes that distinctive mucosal patterns typify experimental cell-mediated (T lymphocyte) reactions in small intestinal mucosa: the Type 1 (infiltrative) lesion, Type 2 (hyperplastic) lesion and the Type 3 (‘destructive’) lesion. |
| Oberhuber et al., 1999 [49] | A modified version of Marsh, revised with subcategories | Subcategorized Type 3 lesions based on villous height as Type 3A mild atrophy, Type 3B marked atrophy and Type 3C total villous atrophy. |
| Corazza and Villanacci, 2005 [50] | A simpler grading system than Marsh, intended to minimize inter-observer disagreement | Grade A: Non-atrophic, with normal crypt and villus architecture and increased IELs (>25 IELs per 100 enterocytes). Grade B1: Atrophic, with villus-to-crypt ratio <3:1, but villi are still detectable and IELs are increased (>25 IELs per 100 enterocytes). Grade B2: Atrophic and flat, villi are not detectable and increased IELs are noted (>25 IELs per 100 enterocytes). Type 1: Normal villi with IELosis. Corresponds to Marsh Type 1, also present in the Oberhuber classification, and to grade A in Corazza & Villanacci’s proposal. Type 2: Shortened villi (<3:1 or <2:1 duodenal bulb) with IELosis and crypt hyperplasia. Corresponds to Types 3A and 3B in the Oberhuber classification and to grade B1 in Corazza & Villanacci’s proposal. Type 3: Completely flat mucosa with IELosis and crypt hyperplasia; corresponds to Marsh Type 3, and also to Type 3C in the Oberhuber classification, and to grade B2 in Corazza & Villanacci’s proposal. |
| Ensari, 2012 [51] | Author argues that duodenal biopsies have almost entirely replaced capsule biopsies of jejunal mucosa for the diagnosis of celiac disease and that the histological scoring systems need to be updated accordingly | The authors argue that, in contrast to scoring systems, histological morphology provides continuous data that may be advantageous in practice and for clinical studies. |
| Taavela et al., 2013 [52] | The authors argue that, in contrast to scoring systems, histological morphology provides continuous data that may be advantageous in practice and for clinical studies. | The tool is based on the quantitative morphological (villus-height-to-crypt-depth ratio; VH:CrD) and inflammatory (density of IEL) variables. Specimens must be rigorously assessed for optimal orientation. |

IEL = intra-epithelial lymphocyte.
modified (Oberhuber), Corazza & Villanaci, and ‘morphometry’ (Table 1) [47–52].

The known histology outcome instruments have limited inter-observer reliability and it is unclear whether the addition of immunohistochemical staining for intra-epithelial lymphocytes is always helpful (except in cases of suspected enteropathy-associated T-cell lymphoma) [53]. The context in which these histology instruments are used also needs to be considered; for example, the task of measuring histological deterioration—or lack of deterioration—in response to a gluten challenge in healed patients is easier than the determination of histological improvement after institution of a GFD. In the first scenario, histological changes may be evident as early as two weeks of after initiation of the gluten challenge [54]. As a result, histological distinction between patients treated with an active agent or placebo might also be expected to occur within that timeframe, with additional distinction over the following weeks.

Regardless of which histological outcome instrument is chosen for a primary analysis, morphometric data, such as villus-height-to-crypt-depth ratio [52], should probably also be collected for exploratory and sensitivity analysis. To optimize accuracy, the histological evaluation should take advantage of unbiased central reading with two independent readers. Cost-effective and scientifically rigorous approaches would include the use of central reading, proper collection of the biopsy material by the site endoscopist (location and number) and accurate specimen handling [55, 56].

Serology

Serological biomarkers are commonly used for the diagnosis of celiac disease in individuals who have not adhered to a gluten-free diet. Once patients do adhere to such a diet, the biomarker levels slowly decrease. Single measurements of celiac serologies, including serum anti-tTG IgA and anti-deamidated gliadin peptide (DGP) IgG antibodies, are generally viewed as not being particularly useful in assessing compliance with a gluten-free diet; however increases over time, especially in the context of a standardized gluten challenge, could be much more informative. There are currently limited data on their kinetics following a gluten challenge and any increase in serological markers may be delayed, compared with changes in histology [57].

Other tests

Intestinal permeability may be higher than normal in celiac disease patients [58], and quantified by the urinary lactulose/mannitol (LAMA) fractional excretion ratio. This test may be particularly relevant to drugs such as Alba’s larazotide acetate, a tight junction regulator; however, LAMA fractional excretion is very variable from person-to-person and could not differentiate between larazotide and placebo in a recent trial [27]. The authors suggested that differences in the method of collecting of urine, compared with the method used in a previous proof-of-concept (POC) study [59], may have been relevant.

As mentioned earlier, the presence of gluten-reactive T cells in celiac disease can be detected using a cytokine release assay, following either a standardized gluten challenge or a challenge with principal gluten peptides, with any correlation to histological changes currently unknown [14].

Gene expression

Genetic biomarkers, specifically expression changes in the intestinal or duodenal mucosa, may be useful early biomarkers and expression changes in genes involved in regulating tight junctions and other adhesion molecules show promise [60].

Endpoints used in recent clinical trials

We conducted a systematic review of all clinical trials listed on the World Health Organisation (WHO) International Clinical Trials Registry Platform [61], which collates information from clinicaltrials.gov and other regional and national clinical trials registries, using the search terms “celiac” and “coeliac”. We identified all celiac disease drug trials with endpoint information.

Table 2. Endpoints employed in recent celiac disease clinical drug trials

| Endpoint                                      | Entry in Clinical Trial Registry                                                                 |
|-----------------------------------------------|---------------------------------------------------------------------------------------------------|
| Intestinal permeability                       | http://clinicaltrials.gov/show/NCT00362856                                                        |
|                                               | http://clinicaltrials.gov/show/NCT00386165                                                        |
|                                               | http://clinicaltrials.gov/show/NCT01257620                                                        |
|                                               | http://clinicaltrials.gov/show/NCT00492960                                                        |
|                                               | http://clinicaltrials.gov/show/NCT00889473                                                        |
|                                               | http://clinicaltrials.gov/show/NCT00859391                                                        |
|                                               | http://clinicaltrials.gov/show/NCT01396213                                                        |
|                                               | http://clinicaltrials.gov/show/NCT01765647                                                        |
| Clinical index of celiac disease activity     | http://clinicaltrials.gov/show/NCT00540657                                                        |
|                                               | http://clinicaltrials.gov/show/NCT00620451                                                        |
|                                               | http://clinicaltrials.gov/show/NCT01661933                                                        |
|                                               | https://www.clinicaltrialsregister.eu/ctr-search/search?query=eudract_number:2013-003660-31       |
|                                               | https://www.clinicaltrialsregister.eu/ctr-search/search?query=eudract_number:2010-023127-23       |
|                                               | https://www.clinicaltrialsregister.eu/ctr-search/search?query=eudract_number:2009-012221-10        |
|                                               | https://www.clinicaltrialsregister.eu/ctr-search/search?query=eudract_number:2007-003450-28       |
| Villus-height-to-crypt-depth ratio            | http://clinicaltrials.gov/show/NCT00962182                                                        |
|                                               | http://clinicaltrials.gov/show/NCT01917630                                                        |
| Histological scoring or not further specified | http://clinicaltrials.gov/show/NCT00671138                                                        |
|                                               | http://clinicaltrials.gov/show/NCT00959114                                                        |
|                                               | http://www.anzctr.org.au/ACTRN12609000738224.aspx                                                |
| Changes in serology/biomarkers               | http://clinicaltrials.gov/show/NCT00962182                                                        |
|                                               | http://www.anzctr.org.au/ACTRN12613001331729.aspx                                                |
| Gluten concentration                         | http://clinicaltrials.gov/show/NCT02060864                                                        |
The results are given in summary form by closely related or identical endpoints with trial numbers and URLs pointing to the trial where the respective endpoint is used. None of the trials are currently in Phase 3; most are at Phase 2 and a few Phase 1 trials have also explored efficacy endpoints (Table 2).

**Other protocol considerations**

Other considerations in protocol design include:

1. **Duration of gluten-free diet**: if the trial design includes a gluten challenge, significant mucosal healing must have occurred first. Requiring a GFD for periods of less than six months may be a risky strategy. There are two reasons for this: patients may not have learnt how to adhere to a GFD and one month may not be long enough to effect any changes. Trial participants should not be newly diagnosed, but should already have learnt how to adhere to a gluten-free diet over 6–12 months.

2. **Compliance with GFD**: a standardized interview with a skilled dietician is widely recommended; however, a recent pilot study of about 400 patients did not show an association between involving a dietician and severity of symptoms, adherence, or quality of life.

3. **Placebo arm**: a protocol with a placebo arm can be the best way to demonstrate a treatment effect, if one exists. In celiac disease, this would be unlikely to represent an ethical issue, at least in adults, since the illness is not life-threatening, usually does not cause major pain, and does not affect development. In pediatric patients, however, nutritional deficiencies may be more serious and a placebo arm might, depending on the length of the study, not be appropriate. One approach might be to have a gluten-free arm and one where gluten is re-introduced along with the investigational therapy.

4. **Baseline endoscopy**: The timing of this step needs to be considered carefully in view of the different time dynamics of changes in the biomarkers obtained (e.g. histology vs. expression analysis). Endoscopy is also important for exclusion patients who still have significant disease activity after having been on a GFD.

**Recommendations for endpoints in clinical trials of drugs for celiac disease**

Phase 2 POC trials offer more flexibility on outcome measures, needing only to provide stakeholders with evidence to make a go/no-go decision. Ideally, this outcome instrument should also inform the development of potential endpoints in Phase 3 trials. To be truly useful, even in the POC stage, POC endpoints need to be able to reasonably predict ‘clinical benefit’, that is, in practical terms, correlate with outcome instruments acceptable for future registration trials. POC endpoints also need to be statistically efficient, i.e. take advantage of as much information as the data contain and be suitable for the most powerful hypothesis tests. Typically, this would favor continuous data over ordinal data or scores.

**Phase 2 proof-of-concept**

Here, the primary endpoint should focus on objective outcomes that can be measured on a continuous scale—such as histological morphometry—rather than as a subjective histology score. It would be worth exploring the potential of newer serological biomarkers—including auto-antibodies, IgA anti-tTG, IgA anti-EMA, IgA anti-DGP, and REG 1α—along with gene expression in the gut mucosa [63]. Serial measurements of transthyretin (pre-albumin), a protein reduced in malabsorption, were reported as an indicator of mucosal recovery in celiac disease in 2001 [64], and may be used as a non-invasive test but further validation of this approach has, to our knowledge, not been pursued. REG 1α, a molecule involved in the regeneration after autoimmune insults could, according to Vives-Pi et al. [63], reflect mucosal recovery and could be useful as a serum biomarker. These authors state that changes in its concentration arise quickly and can be detected before specific autoantibodies are produced after the adaptive immune response has occurred.

Other (exploratory) endpoints should be chosen in Phase 2 according to their ability to inform the design of Phase 3 trials. Engagement of regulatory authorities is important, even at this stage.

**Phase 3 registration trials**

Sponsors should consult with the relevant regulatory authorities long before the end of Phase 2 trials to gain insights into—and perhaps agreement on—appropriate primary endpoints for a Phase 3 trial. A PRO instrument will probably be required, in conjunction with a more ‘objective’ biomarker, probably histology. The biomarker could be a component of a co-primary endpoint together with the PRO, or perhaps a first-ranked secondary endpoint with a PRO instrument being the primary endpoint.

**Table 3. A partnership model for rational drug development to treat eosinophilic esophagitis (adapted from Fiorentino et al. [38]): a framework for similar trials in celiac disease**

| Define EoE | Assess EoE natural history | Identify EoE assessment tools |
|------------|---------------------------|-------------------------------|
| • Unify diagnostic criteria | • FDA and academia collaboration | • Address the importance of EoE-specific COAs |
| • Use symptomatic and histological criteria | • Pool multiple patient registries | • Raise questions on using general terms, such as dysphagia |
| Invite all stakeholders | Standardize data entry | • Identify the need for different COAs for pediatric and adult patients |
| • Discuss overall plan | • Interpret data from different sources | Evaluate intra-epithelial mucosal eosinophilia as a biomarker |
| • Identify key issues | • Recognize EoE subpopulation | |
| • Lack of well-defined and reliable COA | • Define differences between pediatric and adult patients | |

COA — clinical outcome assessment; EoE — eosinophilic esophagitis; FDA: U.S. Food and Drug Administration.
Specifically, the ratio of the villus height to crypt depth could be a suitable biomarker because of its continuous nature. This metric seems to be less subject to inter-observer variation and statistically more informative than other scoring systems. Even if a seemingly simple metric such as villus-to-crypt ratio is chosen, the reading process must be carefully standardized, for example, as described by Taavela et al. [52]. Another issue that should concern drug developers and regulators alike is mucosal healing. Complete mucosal healing is increasingly considered to be a prerequisite for improved long-term outcomes in inflammatory bowel disease (as opposed to mere symptom control) [65] and there is parallel evidence in celiac disease that non-healing, i.e. persistence of villous atrophy, correlates with a higher risk of lymphoproliferative malignancy [66]. When patients with celiac disease can tolerate gluten intake thanks to drugs that keep symptoms at bay—perhaps because inflammatory injury and repair mechanisms are kept in balance—should we not still need to worry about long-term consequences? An ideal agent would help control symptoms and prevent acceleration of inflammation.

The choice of the right PRO instrument is a more difficult problem and experience in drug trials is limited. The greatest body of experience in this context has been accumulated through patients in Alba Therapeutics’ Phase 2b trial (942 patients, see above) where the “celiac disease domains of the gastrointestinal symptoms rating scale” were used [25].

A review of the FDA’s approach to trials in similar diseases with objective manifestations that have a large subjective component—including eosinophilic esophagitis and inflammatory bowel disease—could be useful in framing the discussion of endpoints for celiac disease drug trials. For example, Fiorentino et al. provide a scheme for an evolving partnership model for rational drug development (Table 3) [38].

For eosinophilic esophagitis, clinicians and investigators are still debating the most appropriate clinical endpoints to define therapeutic response [67]; the situation in celiac disease will be no different but drug development still needs to continue, even in the absence of complete agreement.

**Conclusion**

Research into celiac disease has entered an exciting phase: for the first time there may be alternatives to the gluten-free diet. As drugs move out of the proof-of-concept stage to Phase 3 confirmatory trials, they need to be evaluated by endpoints that are tailored to the drug, disease, and target patient population. Perhaps most importantly, these endpoints need to be acceptable to regulatory agencies. We have attempted to open a discussion of the issues that we find are relevant and important, which we hope academic researchers, industry and regulators will continue.

**Conflict of interest statement**: Drs. Gottlieb, Dawson and Hussain work for Quintiles, a company that provides bio-pharmaceutical development services and consulting. They have no other relevant disclosures.

Joseph A. Murray has consulting arrangements with AMAG Pharmaceuticals, Entera Health, Inc, Sonomaceuticals, LLC, BioLineRx and is on the Alvine Pharmaceuticals, Inc. advisory board. Dr. Murray has received grants or research support from Alvine Pharmaceuticals, Inc., and Alba Therapeutics.

**Notes**

1. [http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm370262.htm](http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm370262.htm)

2. [http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf](http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf)

3. [http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf](http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf)

4. [http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm370262.htm](http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm370262.htm)

5. [http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm370262.htm](http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DrugDevelopmentToolsQualificationProgram/ucm370262.htm)

6. [http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf](http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf)

7. Authors’ definition and concept

8. [http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf](http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf)

9. [http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf](http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf)

10. [http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf](http://www.fda.gov/downloads/Training/ClinicalInvestigatorTrainingCourse/UCM283378.pdf)

11. [http://www.fda.gov/drugs/developmentapprovalprocess/drugdevelopmenttoolsqualificationprogram/ucm370262.htm](http://www.fda.gov/drugs/developmentapprovalprocess/drugdevelopmenttoolsqualificationprogram/ucm370262.htm)

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Celiac disease drug development

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Appendix

Glossary

Biomarker. A physiological, pathological, or anatomical patient characteristic that is measured by an automated process or algorithm as an indicator of normal biological processes, pathological processes, or biological responses to a therapeutic intervention. ¹ Composite endpoint.²

A single measure of effect, based on a combination of individual endpoints. These may combine patient-, observer- and clinician-reported outcomes with biomarkers. Particularly useful for drugs that can benefit patients in several ways or if component events are infrequent. Examples: cardiovascular death or hospitalization for heart failure; major adverse cardiac events (MACE): cardiovascular death, non-fatal myocardial infarction (MI), and non-fatal stroke; “clinical worsening,” may include categorical decline in functioning, worsening symptoms, addition of a new medication, hospitalization due to the disease, death, etc. (HRQOL instruments); often analysed as time to first event, or number of events over the study period.

Co-primary endpoint

Here, a clinical trial has multiple primary endpoints and the intervention is judged to have been effective only if it improves on all of the endpoints. In the case where an intervention is deemed effective if it improves on at least one of multiple endpoints, these are termed ‘alternative primary endpoints’.³ The challenge of co-primary endpoints was also discussed by Chuang-Stein et al. (2007).⁴

Effectiveness. An essential component of the basis for marketing approval of a drug: drugs must be safe and effective to justify approval. Effectiveness is defined as a benefit to patients in how they feel, function, or survive due to treatment with the drug.⁵

Endpoint. The way an assessment will be used as a study result and statistically compared between treatment groups to assess the effect of treatment. Endpoints are often named by the assessment measured, but a complete statement of the endpoint should include a full description of what data are collected and how they are analysed to support a specific study objective.⁶

First-ranked secondary endpoint. There is usually only one primary endpoint; if there are more than one, they are linked as co-primary endpoints with a logical AND. Very rarely alternative primary endpoints are used (logical OR). The next endpoint in
the trial design hierarchy is the first-ranked secondary endpoint. Its principal role is to lend support to the primary endpoint. If both are met, results are considered to be more robust than if only the primary endpoint is positive. Sponsors will typically argue that co-primary endpoints are hard to meet and suggest that a co-primary dismantled into one primary and the first-ranked secondary endpoint. This approach allows for the trial to be successful, even if only the primary endpoint was reached. If the first-ranked secondary was not quite met but also pointed in the right direction, chances for regulatory approval are still high7.

**Patient-reported outcome (PRO).** A PRO is a measurement based on a report that comes from the patient (i.e., study subject) about the status of a patient’s health condition without amendment or interpretation of the patient’s report by a clinician or anyone else. A PRO can be measured by self-report or by interview, provided that the interviewer records only the patient’s response. Symptoms or other unobservable concepts known only to the patient (e.g., pain severity or nausea) can be measured only by PRO measures. PROs can also assess the patient’s perspective on functioning or activities that may also be observable by others.8

**Primary endpoint.** All drugs have safety risks; therefore, the only reason that a patient would want to take a drug would be if the drug improved survival, resulted in a benefit that was detectable by the patient (improvement in symptoms, improvement in functional capacity), or decreased the chances of developing a condition or disease complication that is itself apparent to the patient and is undesirable (e.g., stroke). Therefore, a primary endpoint should be a direct measure of one of these. A primary endpoint should generally not be a measure of something that is not important to the patient (exception: validated surrogate endpoint).9

**Secondary endpoint.** Results that are measured at the end of a study, in addition to the main result (primary endpoint), to see if a given treatment worked. Secondary endpoints can explore other aspects of the treatment.10

**Surrogate endpoint.** A surrogate endpoint is a laboratory measure or a physical sign that is intended to be used as a substitute for a clinically meaningful endpoint. Ideally, the surrogate should exist within the therapeutic pathway, between the drug and meaningful benefit, i.e., the drug results in the therapeutic benefit by virtue of its effect on the surrogate. Changes induced by a therapy on a surrogate endpoint are expected to reflect changes in a clinically meaningful endpoint.11

**Table A1. Results of an informal patent search, 2012–14**

| Patent title | Patent number | Abstract | Pub. date | Assignee |
|--------------|---------------|----------|-----------|----------|
| Peptides having protective effect towards the inflammatory activity of peptide 31–43 of α-gliadin in celiac disease | EP2758423 A2 | Intended for preventive and therapeutic purpose by administration to subjects at high risk of developing celiac disease and/or celiac subjects just before a gluten containing meal is ingested. | July 30, 2014 | Istituto Superiore di Sanità, CRA Consiglio per la Ricerca e la sperimentazione in Agricoltura |
| Methods and pharmaceutical compositions for treating celiac disease and gluten intolerance | EP2736525 A1 | Oral administration of ALV003 may protect celiac disease patients and patients otherwise suffering from gluten intolerance from the harmful effects of ingesting food containing gluten. | June 4, 2014 | Alvine Pharmaceuticals, Inc. |
| Treatment of celiac disease with IgA | US8709413 B2 | Oral administration of an IgA or an IgM to the subject suffering from food allergy or food intolerance. | April 29, 2014 | Michael R. Simon |
| Compositions and methods for treating celiac sprue disease | EP2718434 A2 | This invention covers various polypeptides. | April 16, 2014 | University of Washington, through its Center for Commercialization |
| Dietary management of celiac disease and food allergy | US20130344042 A1 | Compositions and methods for dietary management of celiac disease and food allergy via enteral administration of at least one hydrolyzed protein and Lactobacillus rhamnosus GG (LGG). | Dec 26, 2013 | Gretchen Tanbonliong |
| Methods of treating celiac disease | US20130323223 A1 | Compositions which include digestive enzymes and which are formulated to reduce one or more symptoms of celiac disease or a related disorder. | Dec 5, 2013 | Curemark, LLC |
| Compositions and methods for the therapy of inflammatory bowel disease | CA2522957 C | Examples for celiac and other diseases comprise anti-type 1 interferon antagonists, as well as polypeptides and small molecules that inhibit the interaction of Type 1 interferon with its receptor (IFNAR). | Oct 22, 2013 | Medarex, Inc., Lesley B. Pickford, Christopher R. Bebbington, Geoffrey T. Yarranton, David King, Medarex, L.L.C. |
| Use of an immunologically reactive microbial transglutaminase for the diagnosis and/or therapy control of celiac disease or sprue | DE102012007510 A1 | This involves microbial transglutaminase and its immunologically reactive portions or analogues, present in a complex with gliadin. A claim is also included for a diagnostic kit. | Oct 17, 2013 | Aesku.Diagnostics GmbH & Co. KG |
| Patent title | Patent number | Abstract | Pub. date | Assignee |
|--------------|---------------|----------|----------|----------|
| Methods and compositions for treating celiac disease | US20130266584 | The invention features the treatment of gastrointestinal disorders associated with an innate immune response triggered by alpha amylase inhibitor CM3, alpha amylase inhibitor 0.19 (0.19), CM1, CM2, CMa, CMd, CM16, CMb, CMX1/CMX3, CMX2, and/or alpha amylase inhibitor 0.53 (0.53). | Oct 10, 2013 | Detlef Schuppan, Yvonne Junker, Towia Libermann, Simon T. Dillon |
| Compositions and methods for determining celiac disease | US20130109034 | The compositions include recombinant proteins that contain tissue transglutaminase and deamidated gliadin sequences. Also provided is a method to identify an individual as having celiac disease, based on the presence of antibodies. | May 2, 2013 | IMMCO Diagnostics, Inc. |
| Method for treating celiac disease | US20130122086 | Enteric compositions comprising one or more tight junction agonists and/or antagonists are provided. Compositions may include a delayed-release coating. | May 16, 2013 | Alba Therapeutics Corp. |
| Methods to predict risk for celiac disease by detecting anti-flagellin antibody levels | US8409819 | Methods, assays, and kits for predicting or stratifying the risk of celiac disease, based upon HLA-DQ genotype and/or anti-flagellin antibody levels. | April 2, 2013 | Nestec S.A. |
| Methods and pharmaceutical compositions for treating celiac disease and gluten intolerance | WO2013016427 | Methods for protecting a subject from a deleterious effect of gluten ingestion, including oral administration of ALV003. | Jan 31, 2013 | Alvine Pharmaceuticals, Inc. (applicant) |
| Treatment of celiac disease with IgA | US8313730 | A process for inhibiting symptoms of a subject with celiac disease is provided that includes administration of monoclonal-, or polyclonal-, monomeric, dimeric, or polymeric IgA. | Nov 20, 2012 | Michael R. Simon |
| Diagnostic method and breath testing device | US20120234076 | A diagnostic method and breath-testing device for the diagnosis of celiac disease, using a hydrogen-selective sensor in the form of a ZnO nanowire-based sensor fabricated using a focused ion beam (FIB/SEM) instrument or a thin film. | Sept 20, 2012 | Anastasia Rigas |
| New markers for the diagnosis of celiac disease | US20120225442 | New peptides and to their use in the diagnosis of celiac disease. | Sept 6, 2012 | Karl Skriner |
| Epitopes related to coeliac disease | EP2486935 | Epitopes that are useful in methods of diagnosing, treating, and preventing coeliac disease and methods of using and detecting these epitopes. | Aug 15, 2012 | BTG International Limited (applicant) |
| Compositions and methods for treatment of celiac disease | EP2367561 | Agents and vaccines for treating and diagnosing celiac disease. In particular, a combination of three peptides that are useful for treating and diagnosing celiac disease in a large proportion of patients.\(^1\) | June 6, 2012 | Immusant Inc |
| Testing efficacy for celiac disease | US20120107847 | A method to determine effectiveness of a compound or composition in treatment of celiac disease or gluten intolerance. | May 3, 2012 | Dsm Ip Assets B.V. |
| Materials and methods for the treatment of celiac disease | US20120076861 | Materials and methods for the treatment of celiac disease and monitoring the treatment of such subjects. | March 29, 2012 | University Of Maryland, Baltimore, Alba Therapeutics Corporation |
| Treatment of celiac disease with IgA | US8119104 | A process for inhibiting symptoms of celiac disease that includes administration of monoclonal-, or polyclonal-, monomeric, dimeric, or polymeric IgA. | Feb 21, 2012 | Michael R. Simon |