Safety and efficacy of AMG 714 in adults with coeliac disease exposed to gluten challenge: a phase 2a, randomised, double-blind, placebo-controlled study

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Summary

Background Interleukin 15 (IL-15) is implicated in the pathophysiology of coeliac disease. AMG 714 is the first anti-IL-15 monoclonal antibody to be investigated for the treatment of coeliac disease. We aimed to investigate the effects of AMG 714 in patients with coeliac disease who underwent gluten challenge.

Methods This randomised, double-blind, placebo-controlled, parallel-group, phase 2a trial was done at three clinical sites in Finland. Inclusion criteria included age 18–80 years, a confirmed diagnosis of coeliac disease, and adherence to a gluten-free diet for at least 12 months before screening. Patients were randomly assigned (1:1:1) to 150 mg AMG 714, 300 mg AMG 714, or placebo using permuted blocks and stratified by study site and sex. Patients and study staff were masked to treatment assignment. Treatments were administered by two subcutaneous injections every 2 weeks for 10 weeks (total six doses). Patients without severe villous atrophy at baseline received a gluten challenge (2–4 g daily) during weeks 2–12. Small bowel biopsy samples were obtained for histological assessments at baseline and week 12. The primary efficacy endpoint was the percentage change from baseline to week 12 in villous height-to-crypt depth (VHCD) ratio. Secondary endpoints were CD3-positive intraepithelial lymphocyte density; clinical symptoms measured by gastrointestinal symptom rating scale (GSRS), coeliac disease GSRS, and Bristol stool form scale (BSFS); and changes in anti-tTG and anti-DGP antibodies from baseline. The primary analysis was done in the per-protocol 1 population of patients who received at least one dose of study drug and who underwent the gluten challenge. Safety analyses were done in all patients who received at least one dose of study drug. This trial is registered at ClinicalTrials.gov, NCT02637141 and EudraCT, 2015-003647-19.

Findings Between April 13, 2016, and Nov 22, 2016, 64 patients were enrolled and randomly assigned to either the 150 mg AMG 714 group (n=22), the 300 mg AMG 714 group (n=22), or the placebo group (n=20). Two patients did not start treatment and two did not provide post-treatment biopsy samples. 49 patients underwent the gluten challenge (per-protocol 1 population) and 11 patients did not because of baseline villous atrophy. AMG 714 did not prevent mucosal injury due to gluten challenge. The least square mean difference in the relative change from baseline in VHCD ratio was –2·49% (95% CI –16·82 to 11·83; p=0·73) between 150 mg AMG 714 and placebo and 6·39% (–7·07 to 19·85; p=0·34) between 300 mg AMG 714 and placebo. Neither comparison was statistically significant. The density of CD3-positive intraepithelial lymphocytes increased in all groups, with smaller increases in the 300 mg group (–41·24% [95% CI –79·28 to –3·20] *vs* placebo, nominal p=0·03) but not the 150 mg group (–14·32% [–54·39 to 25·74], nominal p=0·47). Clinical symptoms were ameliorated with AMG 714 treatment between baseline and week 12, particularly diarrhoea as measured by the BSFS (nominal p=0·01 for 150 mg *vs* placebo, and nominal p=0·0002 for 300 mg *vs* placebo). Serum antibody titres for anti-tTG and anti-DGP antibodies increased in all three treatment groups, with no significant difference between AMG 714 and placebo. Treatment-emergent adverse events occurred in 21 (95%) patients in the 150 mg AMG 714 group, 0 (95%) in the 300 mg AMG 714 group, and 19 (100%) in the placebo group. The most common treatment-emergent adverse events were gastrointestinal disorders (17 [77%] participants in the 150 mg AMG 714 group, 16 [76%] in the 300 mg AMG 714 group, and 13 [68%] in the placebo group). Injection site reactions were the most common individual adverse event, reported in eight (36%) patients in the 150 mg AMG 714 group, 11 (52%) in the 300 mg group, and five (26%) in the placebo group. No serious adverse events occurred.

Interpretation The primary endpoint, change in VHCD ratio from baseline after 12 weeks of treatment in patients with coeliac disease undergoing gluten challenge, was not significantly different between placebo and AMG 714 at either 150 mg or 300 mg. Effects on intraepithelial lymphocyte density and symptoms suggest that further research of AMG 714 may be warranted in patients with non-responsive coeliac disease.

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Research in context

Evidence before this study

No approved drug therapy exists to treat coeliac disease and patients adhering to a gluten-free diet remain at risk of being exposed to gluten through contamination. Interleukin 15 (IL-15) has been strongly implicated in the pathology of coeliac disease. We searched PubMed using the terms "interleukin 15", "anti-IL-15", and "celiac" for articles published in English up to Sept 10, 2018. No clinical trials have been done to assess any antiIL-15 treatment for coeliac disease. A study of anti-IL-15 antibody in gluten-sensitive rhesus monkeys with coeliac disease showed potential efficacy.

Added value of this study

This study provides proof of mechanism and proof of concept for an anti-IL-15 monoclonal antibody as a treatment for patients with coeliac disease who are exposed to gluten. The results suggest that the treatment can attenuate gluten's damaging effects on intestinal inflammation and this finding was corroborated by clinical symptoms observations.

Implications of all the available evidence

Our findings confirm that IL-15 plays an important role in coeliac disease and suggest that the inhibition of IL-15 is a viable strategy in the treatment of coeliac disease. AMG 714 (PRV-015) merits further assessment in a longer and larger dose-finding study in patients with non-responsive coeliac disease despite being on a gluten-free diet.

Introduction

Coeliac disease is a systemic autoimmune disease triggered by gluten consumption in genetically susceptible individuals.[1] About 1% of the European and North American population is affected by coeliac disease, although many patients remain undiagnosed because of insufficient awareness and highly variable presentation of the disease. The pathophysiology of coeliac disease is characterised by an abnormal immune response to gluten, a composite of various proteins found in wheat, barley, and rye. The proteins in gluten belong primarily to the two classes known as gliadins and glutenins. Gluten is very common in processed foods and is well tolerated by most people. In patients with coeliac disease, however, gluten elicits innate and adaptive immune responses, causing T-cell activation and initiating an inflammatory cascade that ultimately leads to mucosal damage in the small intestine.

Coeliac disease is diagnosed through a combination of serology tests (including tests for antitissue transglutaminase [anti-tTG] antibodies, endomysial antibodies, and antideamidated gliadin peptide [anti-DGP] antibodies) and clinical assessments, with confirmation by histology in biopsy tissues obtained via upper endoscopy.[2] Gluten-triggered histological lesions in coeliac disease appear on a continuum from normal to flat in small intestine epithelium, and the flat form is characteristic of the hyperplastic crypt end stage of the injury. Architectural changes in the mucosa can be measured using the villus height-to-crypt depth (VHCD) ratio.[3,4] A lower VHCD ratio indicates a flattening of the mucosa and more severe intestinal injury. The VHCD ratio is used in the diagnosis and monitoring of coeliac disease and is a common research tool in coeliac disease to asses disease activity.[3–7]

Coeliac disease causes debilitating symptoms and serious medical complications, because damage to the small intestine can lead to nutrient malabsorption and subsequent clinical manifestations, such as anaemia, osteopenia, weight loss, and insufficient growth in children. Additionally, extraintestinal symptoms and systemic manifestations are often present, including dermatitis, infertility, and neurological and skeletal disorders.[1] The stimulation of intestinal lymphocytes over time can lead to serious complications such as refractory coeliac disease and enteropathy-associated T-cell lymphoma.[8] Mortality is increased in patients with coeliac disease and persistent intestinal mucosal damage.[9]

The only available management option for coeliac disease is lifelong total avoidance of gluten—ie, a gluten-free diet. Although simple in theory, maintaining a strict gluten-free diet is difficult in practice because of occasional dietary transgressions and gluten contamination in foods purported to be gluten free. At least half of all diagnosed patients who are following a gluten-free diet are estimated to continue to have some disease activity, because as little as 50 mg per day of gluten exposure may trigger immune activation and mucosal damage.[10–15] Patients who continue to have symptoms despite following a gluten-free diet, due to exposure to unintentional and variable amounts of gluten, are considered to have non-responsive coeliac disease. To meet the medical needs of patients with coeliac disease, a treatment beyond gluten-free diet is urgently needed.

The proinflammatory cytokine interleukin 15 (IL-15) has been identified as a major mediator in the pathophysiology of coeliac disease.[16,17] IL-15 is produced in the small intestine by antigen-presenting cells and epithelial cells and is an essential, non-redundant factor for the activation and proliferation of intraepithelial lymphocytes. The intraepithelial lymphocytes, primarily CD8 cells, destroy intestinal epithelial cells and cause the villous atrophy that is characteristic of coeliac disease.[18,19] Numerous genetic variants in the IL-15 and IL-15 receptor α (IL-15RA) genes have been associated with IL-15 protein expression and the risk of coeliac disease.[20] Additionally, IL-15 renders effector T cells resistant to inhibition by regulatory T cells,[19] thus promoting the loss of tolerance to food

antigens.[18,21] Further, IL-15 induces an increase in enterocyte apoptosis, which in turn correlates with mucosal damage and villous atrophy.[22]

AMG 714, a fully human immunoglobulin monoclonal antibody (IgG1κ), binds to and inhibits the function of IL-15 in all of its forms (cis, trans, and soluble IL-15 bound to IL-15RA), and blocks IL-15-induced T cell proliferation.[23] It has also demonstrated dose-dependent inhibition of IL-15-induced TNFα production. Thus, AMG 714 is a plausible candidate for the treatment of coeliac disease.

In this phase 2a, proof-of-principle study, we aimed to assess the efficacy and safety of 150 mg and 300 mg AMG 714 in patients with coeliac disease who had been on a gluten-free diet. As patients on a gluten-free diet might have unexpected and variable amounts of gluten exposure in the real world, gluten challenge with a controlled daily consumption of 2–4 g of gluten was given to most enrolled patients who could tolerate it. This approach allowed us to investigate the clinical and biophysiological activities of AMG 714 against coeliac disease in less time than would have been possible under the natural condition of real-world gluten exposure.

Methods

Study design

This was a randomised, double-blind, placebo-controlled, parallel-group, phase 2a trial to assess the efficacy and safety of AMG 714 (now named PRV-015) 150 mg and 300 mg compared with placebo in adults with coeliac disease. The study was done at three clinical sites in Finland. The study protocol was approved by the ethics committee of the Tampere University Hospital District (Tampere, Finland) for all three study sites. The study conduct and monitoring followed good clinical practice guidelines of the International Council for Harmonisation and were in accordance with the Declaration of Helsinki.

Participants

Adults aged 18–80 years who had a confirmed diagnosis of coeliac disease and had been adhering to a gluten-free diet for at least 12 months before screening were considered for inclusion. Self-report of adherence to a gluten-free diet was assessed by investigators and corroborated by negative serology results (test for anti-tTG antibodies). Other inclusion criteria were coeliac disease-compatible HLA-DQ genotype (DQ2 or DQ8); body-mass index of 16–45 kg/m²; adequate haematological, renal, and hepatic laboratory parameters; and negative anti-tTG IgA (demonstrating attempted adherence to the gluten-free diet) and *Helicobacter pylori* tests. Women of childbearing potential were confirmed as not pregnant and required to maintain birth control from study entry through 6 months after the end of the study.

Patients were excluded if they had a current diagnosis of any severe complication of coeliac disease, such as refractory coeliac disease type I or type II, enteropathy-associated T-cell lymphoma, ulcerative jejunitis, or intestinal perforation; any autoimmune disease other than coeliac disease and dermatitis herpetiformis that might interfere with study participation or require systemic immunomodulation therapy; active disease symptoms, defined as a coeliac disease gastrointestinal symptom rating scale (CeD GSRS) score of more than 2·3 at screening; any other chronic, active gastrointestinal disease such as peptic ulcer, gastroesophageal reflux disease, inflammatory bowel disease, or irritable bowel syndrome; any known symptomatic food allergy; other significant diseases, including but not limited to most types of cancer, heart disease, and pulmonary disease; use of systemic immune suppressants within 3 months or five half lives, whichever is longer, before screening; receipt of a live vaccine within 14 days before the first administration of study drug; self-reported drug or alcohol abuse in the year before screening; hypersensitivity to the study drug, related drugs, or excipients; pregnancy; and participation in another study. Patients were also excluded if they had active or persistent infection that required systemic treatment (antibiotics, antifungal, or antiviral) at screening; active gastrointestinal infection; a history of tuberculosis, hepatitis B, hepatitis C, or HIV; persistent or severe infection within 3 months before screening; or any opportunistic infection within 3 years before screening. To allow for gluten challenge, patients with a history of anaphylactic reaction to wheat or gluten or other ingredients in the gluten products used in the study were also excluded.

All participants provided written informed consent, including permission to perform analyses on collected blood, urine, stool, and small intestine biopsy samples for the purposes of research into disease biomarkers and the pathogenetic mechanisms of coeliac disease.

Randomisation and masking

Eligible patients were randomly assigned in a 1:1:1 ratio to the 150 mg AMG 714, 300 mg AMG 714, or placebo group. Randomisation was done using permuted blocks and stratified by study site and sex. The treatment allocation sequence was generated by the biostatistician. This was a double-blind study. Neither patients nor study staff who were involved in study treatment and assessments were aware of each patient's assigned group throughout the entire study. To maintain masking, each dose was prepared by an unmasked pharmacist in two syringes, and each syringe contained either 150 mg AMG 714 or a placebo solution with matching appearance. Patients in the 150 mg group were each injected with one 150 mg AMG 714 syringe and one placebo syringe; patients in the 300 mg AMG 714 group with two 150 mg AMG 714 syringes; and patients in the placebo group with two placebo syringes.

Procedures

Patients received 150 mg AMG 714, 300 mg AMG 714, or placebo via two subcutaneous injections once every

2 weeks for a total of six doses over 10 weeks (±3 days) from day 0 (week 0). At each visit, AMG 714 or placebo was administered by a qualified study staff member who was masked to treatment assignment.

Each dose was injected into different locations on a patient's anterior abdominal wall. The injections were administered consecutively within 30 s of each other and about 2 cm apart. The side of the abdominal wall used for injections was alternated at every visit (ie, left side at one visit, right side at the next visit).

Patients could be removed from study treatment at the investigator's discretion or patient's own request, because of protocol violation or significant non-compliance, or concomitant medications that were not allowed during study treatment and assessments. Dose reduction was not allowed but dosing could be interrupted as decided by the investigator.

Gluten-free and gluten-containing cookies with the same appearance were dispensed to study patients in a single-blind fashion, so as not to alert them to the identity of the material consumed. The cookies were manufactured by Brander, a specialty gluten-free bakery in Tampere, Finland, in dedicated rooms for gluten-containing or gluten-free products as appropriate. The cookies were Finnish rusks or double-baked cake breads, weighted and packaged in half-day portions. During the study, the cookies were analysed for gluten content by Biomedal (Seville, Spain) to ensure gluten content consistency, and outlier batches were discarded.

Gluten-free cookie consumption began on day 1, 1 day after the first dose of the study drug was administered on day 0, through day 13. Patients were instructed to consume the cookies twice daily, along with two gluten-free main meals as usual, for the first 2 weeks. This placebo gluten period was designed to enable the assessment of psychological effect of consuming products resembling gluten-containing products and to assess the safety of AMG 714 in the absence of a gluten challenge. At the week 2 visit when the second dose of study drug was given (day 14), gluten-containing cookies, each serving containing 1–2 g of gluten, were dispensed to patients for twice daily consumption in the same manner through week 12 (2 weeks after the final dose). If a patient missed a dose of the study-provided gluten product with a meal, the missed dose was to be consumed as soon as possible or with the next main meal. No more than two doses of the gluten-free or gluten-containing cookies were to be consumed in one day. Therefore, the daily dose of gluten intake was 2–4 g from week 2 to week 12.

Study patients' adherence to their gluten-free diet and consumption of the gluten-free or gluten-containing cookies were assessed using iVYLISA, a gluten-specific immunogenic peptide assay for stool (quantitative sandwich enzyme-linked immunosorbent assay) and urine (lateral flow test) samples, which were collected every 2 weeks. These results were not given to study sites or patients during the study. Patients who appeared to have gluten-free diet transgressions or suspected non-compliance to the required gluten consumption were counselled and allowed to continue in the study.

At baseline and at week 12, four biopsy specimens per patient were collected from the second portion of the duodenum via white-light endoscopy. All specimens were shipped to the central laboratory (Jilab, Tampere, Finland) and oriented by coeliac pathology experts.The analyses of VHCD and immunohistochemistry for CD3-positive intraepithelial lymphocyte enumeration were done in a masked manner at a central expert laboratory (Jilab).

Patients recorded their stool consistency daily from baseline to week 16 using the Bristol stool form scale (BSFS); serum concentration of AMG 714 (pharmacokinetics) was measured at baseline and weeks 2, 4, 8, 12, and 16; GSRS and the coeliac-specific subset (CeD GSRS) were measured weekly;[24,25] the coeliac disease patient-reported outcome (CeD PRO) questionnaire (used prospectively for the first time in this study) was done daily;[25] and the physician global assessment (PGA) scale was assessed face-to-face at baseline and weeks 2, 4, 8, 12, and 16.

Safety and clinical assessments were done generally every 2 weeks during study visits. Patient-reported outcomes were recorded on an electronic diary at home throughout the study period. Anti-tTG and anti-DGP antibodies were measured monthly using recombinant human ELISA kits (Inova Diagnostics, San Diego, CA, USA) at a central laboratory. Blood samples of patients who received AMG 714 were initially tested for binding antidrug antibodies using a bridging immunoassay; samples positive for binding antibodies were then tested for neutralising antibodies in a target-binding immunoassay.

Outcomes

The primary efficacy endpoint was the percentage change from baseline to week 12 in VHCD ratio, separately compared between each AMG 714 dose and placebo.

Secondary efficacy endpoints were the density of CD3-positive intraepithelial lymphocytes in the small-intestine biopsy samples;[3] clinical symptoms, measured by GSRS, CeD GSRS, and the BSFS; and changes of anti-DGP and anti-tTG antibodies from baseline. The Marsh score was calculated indirectly by conversion from the VHCD, but is not reported here.

Exploratory endpoints were pharmacokinetics and pharmacodynamics measurements, and biomarkers of disease activity (such as C-reactive protein, serum IL-15, and biopsy tissue mRNA expression profile, which will be reported elsewhere).

Safety endpoints were adverse events, clinical laboratory tests, physical examination, vital signs, and immunogenicity.

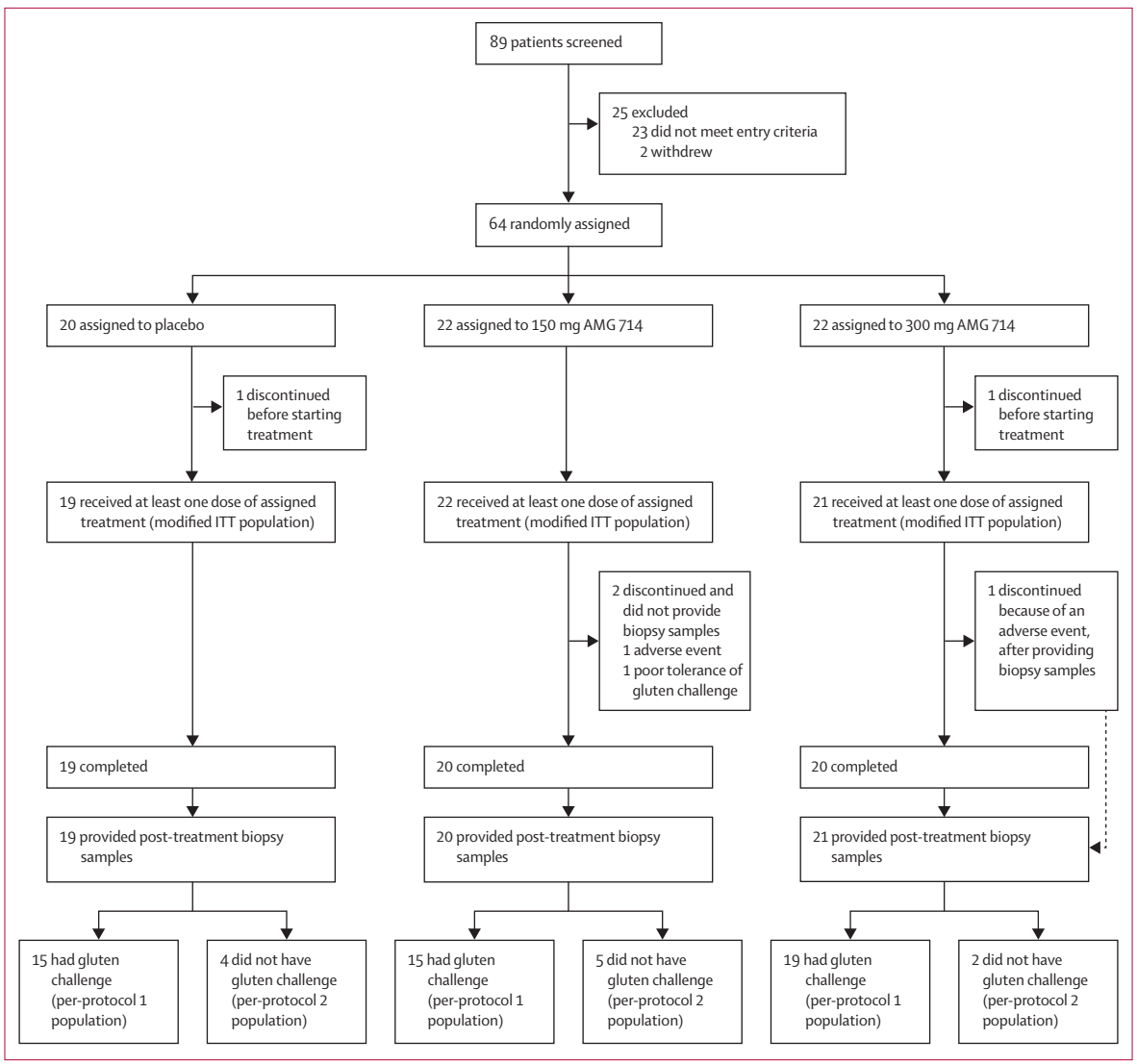

Figure 1: **Trial profile**
ITT=intention to treat.

Statistical analysis

A power calculation showed that a sample size of about 63 patients (21 per treatment group) had 88·8% power to detect a 40-point difference between the placebo and 300 mg AMG 714 group and 79·3% power to detect a 35-point difference between the placebo and 150 mg AMG group, assuming 45% decrease in the placebo group, 35% decrease in the 150 mg group, and 40% decrease in the 300 mg group in the primary endpoint (percent change from baseline to week 12 in VHCD ratio).

The two AMG 714 dose groups (150 mg and 300 mg) were each compared with the placebo group. The primary endpoint was analysed using analysis of covariance (ANCOVA), where the baseline VHCD ratio, site, and sex were included as covariates and treatment

	150 mg AMG 714 group (n=22)	300 mg AMG 714 group (n=21)	Placebo group (n=19)
Age, years			
Mean (SD)	51·0 (15·5)	47·1 (15·2)	55·8 (14·4)
Range	19–71	20–69	24–72
Sex			
Female	16 (73%)	17 (81%)	13 (68%)
Male	6 (27%)	4 (19%)	6 (32%)
Race			
White	22 (100%)	21 (100%)	19 (100%)
Bodyweight, kg	72·3 (12·3)	68·9 (13·8)	78·1 (14·8)
Body-mass index, kg/m²	25·8 (4·5)	24·4 (3·8)	27·1 (4·2)

Data are n (%) or mean (SD) unless otherwise stated.

Table 1: **Demographic and baseline characteristics (intention-to-treat population)**

	150 mg AMG 714 group	300 mg AMG 714 group	Placebo group
Per-protocol 1 population			
Number of patients	15	19	15
Mean (SD) percentage change	−65·25% (19·65)	−55·45% (21·37)	−60·98% (20·21)
Least square mean difference (95% CI) versus placebo	−2·49% (−16·82 to 11·83); p=0·73	6·39% (−7·07 to 19·85); p=0·34	..
Per-protocol 2 population			
Number of patients	5	2	4
Mean (SD) percentage change	−5·98% (79·76)	−2·75% (10·96)	−16·33% (24·00)
Least square mean difference (95% CI) versus placebo	−12·89% (−86·28 to 60·51); p=0·65	51·63% (−34·14 to 137·41); p=0·17	..

Statistical comparison between each AMG 714 dose and placebo was done using analysis of covariance, where the baseline VHCD ratio, site, and sex were included as covariates and treatment group included as a fixed effect. VHCD ratio=villous height-to-crypt depth ratio.

Table 2: **Percentage change from baseline to week 12 in VHCD ratio by treatment group**

	150 mg AMG 714 group	300 mg AMG 714 group	Placebo group
Per-protocol 1 population			
Number of patients	15	19	15
Mean (SD) percentage change	95·25% (59·49)	76·58% (65·55)	104·65% (85·67)
Least square mean difference (95% CI) versus placebo	−14·32% (−54·39 to 25·74); p=0·47	−41·24% (−79·28 to −3·20); p=0·03	..
Per-protocol 2 population			
Number of patients	5	2	4
Mean (SD) change	−4·14% (46·57)	30·60% (40·16)	24·88% (47·38)
Least square mean difference versus placebo	21·87% (−146·44 to 190·17); p=0·74	115·63% (−231·39 to 462·66); p=0·41	..

Statistical comparisons were made using analysis of covariance, where the baseline VHCD ratio, site, and sex were included as covariates and treatment group as a fixed effect. VHCD ratio=villous height-to-crypt depth ratio.

Table 3: **Percentage change from baseline to week 12 in intraepithelial lymphocyte density by treatment group**

group as a fixed effect in the statistical model. Secondary efficacy analyses were done using various statistical methods as appropriate for each endpoint, and the baseline value, site, and sex were also covariates in the analytical models. The relative percentage change from baseline in intraepithelial lymphocyte density was compared between AMG 714 and placebo using ANCOVA. The clinical endpoints (GSRS, CeD PRO, PGA, and BSFS) were analysed using the linear mixed-effect model repeat measurement approach and analysis of variance (ANOVA). A Fisher's exact test was used to compare the proportion of patients with and without disease activity at week 12. Significance was set at an α error level of 0·05. No correction for multiplicity was performed in this small exploratory phase 2a study. All safety variables were summarised by treatment group using descriptive statistics.

The populations for analysis were the per-protocol and intention-to-treat (ITT) populations. The ITT population was a modified ITT population including all randomly assigned patients who received at least one dose of the study drug. The per-protocol population encompassed all randomly assigned patients who received at least one dose of the study drug and were histologically evaluable. Patients who did the gluten challenge were analysed as per-protocol 1. Per-protocol 1 was the primary analysis set for the primary endpoint. Patients who could not receive the gluten challenge due to villous atrophy (VHCD ratio <1·5) at baseline or did so for less than a week because of high gluten sensitivity were the per-protocol 2 population. The per-protocol 1 and per-protocol 2 datasets were mutually exclusive and analysed separately for efficacy endpoints.

All statistical analyses were performed using SAS for Windows, version 9.4. A data and safety monitoring board was responsible for reviewing the safety data and advising on the need for study interruption if cases of severe adverse events occurred. This study is registered at ClinicalTrials.gov, NCT02637141 and EudraCT, 2015-003647-19.

Role of the funding source

Employees of the funder had a role in study design, data collection, data analysis, data interpretation, and in writing of the report. MLL, FL, and MM had full access to all study data and final responsibility for the decision to submit for publication.

Results

We enrolled patients between April 13, 2016, and Nov 22, 2016, and the last patient visit was March 14, 2017. 89 patients were screened, of whom 25 were excluded, so 64 patients were enrolled and randomly assigned to either the 150 mg AMG 714 group (n=22), the 300 mg AMG 714

group (n=22), or the placebo group (n=20). One patient in the placebo group and one in the 300 mg AMG 714 group did not receive any study drug; thus, the ITT population consisted of 19 patients in the placebo group, 22 in the 150 mg AMG 714 group, and 21 in the 300 mg AMG 714 group (figure 1). Two patients, both in the 150 mg group, discontinued and did not provide a post-treatment biopsy sample for analysis and therefore were not included in the per-protocol population, resulting in 49 patients in the per-protocol 1 and 11 patients in the per-protocol 2 datasets. Of the 64 randomised patients, 59 (92%) completed the study; two patients in each of the AMG 714 dose groups and one patient in the placebo group withdrew, including one patient in each of the AMG 714 dose groups who withdrew because of adverse events.

For the 59 (92%) patients who completed the study, the duration of follow-up was the protocol-mandated 16 weeks after the initiation of study treatment. Three patients discontinued from the study at 8, 56, and 85 days after the first dose.

The demographic and baseline characteristics were similar across the three treatment groups (table 1). Of the 62 patients in the ITT population, 46 (74%) were women, which is consistent with the gender distribution of the population of patients with coeliac disease. The mean age was 51 years (SD 15). The mean bodyweight was 78 kg (SD 15) in the placebo group, 72 kg (12) in the 150 mg AMG 714 group, and 69 kg (14) in the 300 mg AMG 714 group. The mean time since coeliac disease diagnosis was 16 years in per-protocol 1 and 8 years in per-protocol 2. All patients were white.

Gluten tests of stool and urine samples collected throughout the study revealed that patients in per-protocol 1—ie, those who did the gluten challenge—were compliant with the protocol-required gluten consumption during weeks 2–10, and that compliance began to decrease during weeks 10–12 (appendix p 1). Notably, patients in per-protocol 2—ie, those who did not receive gluten challenge because of baseline mucosal damage—showed some positive gluten stool samples at the screening and baseline visits and during weeks 8–12, indicating that there was gluten exposure in their food intake despite attempted adherence to a gluten-free diet.

In the per-protocol 1 population, all three treatment groups showed gluten-induced mucosal injury, as evidenced by the relative decrease in VHCD ratio from baseline to week 12 (table 2). The least square mean difference between 150 mg AMG 714 and placebo was –2·49% (95% CI –16·82 to 11·83; p=0·73). The least square mean difference between 300 mg AMG 714 and placebo was 6·39% (95% CI –7·07 to 19·85; p=0·34). Neither was statistically significant.

In the per-protocol 1 population, all three groups showed a relative increase in intraepithelial lymphocyte density because of the gluten challenge, which was consistent with the observed decrease in VHCD ratio (table 3). The least square mean difference between 150 mg AMG 714 and placebo was –14·32% (95% CI –54·39 to 25·74; nominal p=0·47). By contrast, the 300 mg dose showed a least square mean difference of –41·24% (–79·28 to –3·20) compared with placebo, with a nominal p value of 0·03, indicating attenuation of the effect of gluten on intraepithelial lymphocyte density. The individual patients' changes from baseline in both histological endpoints are in the appendix (p 2).

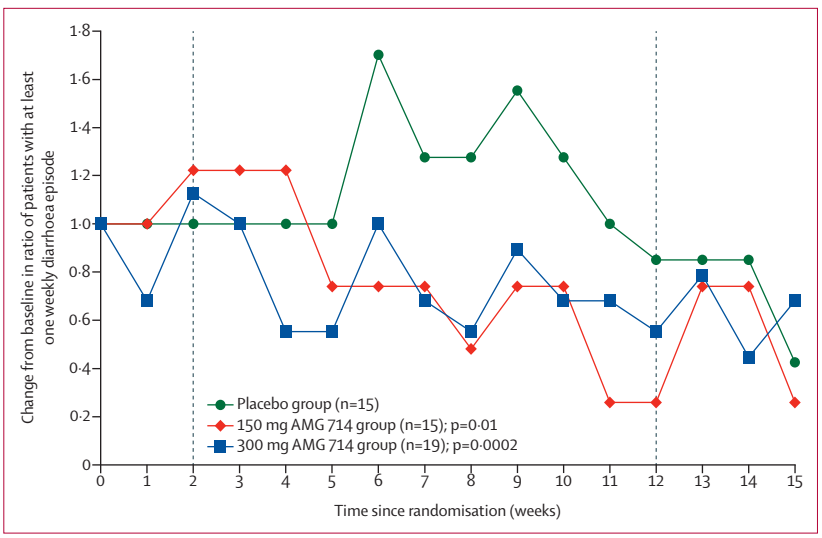

Figure 2: **Change from baseline in the relative ratio of the proportion of patients with one or more weekly episodes of diarrhoea (per protocol 1 population)**
Change from baseline was calculated as a ratio: proportion of patients with diarrhoea at post-treatment visit over proportion of patients with diarrhoea at baseline. Diarrhoea was defined as a Bristol stool form scale score of 6 or 7. Comparison between each AMG 714 group and placebo was done by one-way ANOVA. Dotted lines show the period of gluten challenge (week 2 to week 12).

In the per-protocol 2 population (table 2), the small sample sizes do not allow for conclusions to be drawn.

In the per-protocol 1 population, all three groups had increases from baseline in mean weekly CeD GSRS scores during the gluten challenge period, indicating worsening of symptoms, which returned to baseline after week 12 (appendix p 3). These changes were consistent with the histological findings. The least square mean change from baseline over 12 weeks in CeD GSRS score was 2·97 for placebo, 0·96 for 150 mg AMG 714, and 0·46 for 300 mg AMG 714. The least square mean treatment difference was –2·01 (95% CI –4·97 to 0·94) between 150 mg AMG 714 and placebo (p=0·18) and –2·51 (–5·28 to 0·25) between 300 mg AMG 714 and placebo (p=0·074). The results for the broader mean GSRS score, not focused on coeliac symptoms, were similar between placebo and AMG 714 groups (data not shown).

See **Online** for appendix

The proportion of patients with at least one episode of diarrhoea each week, defined as a BSFS score of 6 or 7, increased then decreased during the gluten challenge period in the placebo group but slightly decreased over time in both AMG 714 groups (figure 2). For the change from baseline over the 12 weeks, the comparisons between 150 mg and placebo (p=0·01) and between 300 mg and placebo (p=0·0002) both had nominal p values of less

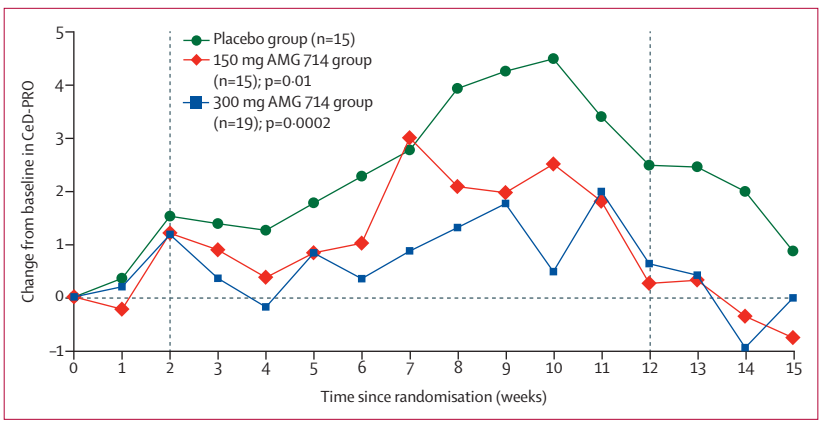

Figure 3: **Change from baseline in CeD PRO scores over time (per-protocol 1 population)**
Change from baseline was analysed using a linear mixed-effects repeated-measures model with the baseline value, treatment group, site, sex, timepoint, and a timepoint-by-treatment group interaction terms as fixed effects with an underlying correlation structure between the time points that resulted in the best fit for the model. Patient was included as a random effect. Dotted lines show the period of gluten challenge (week 2 to week 12). CeD PRO=coeliac disease patient-reported outcome.

than 0·05. The baseline mean number of bowel movements per week was 9·6 (SD 2·9) in the placebo group, 8·9 (3·7) in the 150 mg AMG 714 group, and 10·2 (4·0) in the 300 mg AMG 714 group. At week 12 (end of gluten challenge), the mean number of bowel movements per week was similar between the groups (11·6 [SD 4·0] in the placebo group, 9·3 [2·6] in the 150 mg AMG 714 group, and 11·5 [5·3] in the 300 mg AMG 714 group).

The coeliac disease-related serology results showed gluten challenge-induced seroconversions and increase in serum antibody titres for anti-tTG and anti-DGP antibodies in all three treatment groups, although the anti-DGP IgA response to gluten challenge appeared to be numerically smaller in the 300 mg AMG 714 group, the difference was not significant (appendix pp 4–5). There was no difference between AMG 714 and placebo in the effects on anti-tTG IgA (data not shown).

All groups in the per-protocol 1 population also reported increased mean weekly CeD PRO scores, suggesting worsening of symptoms during the gluten challenge period (figure 3). The least square mean treatment difference in the change of CeD PRO score from baseline between 150 mg AMG 714 and placebo was –1·49 (95% CI –4·00 to 1·02) and not statistically significant (p=0·24), but the difference between 300 mg AMG 714 and placebo of –2·76 (–5·13 to –0·40) had a nominal p value of less than 0·05 (p=0·023), suggesting an attenuation of gluten effects.

The PGA scores at week 12 (end of the gluten challenge) were dichotomised for outcome, where a score of 2 or less (no disease activity) was considered a treatment success and a PGA score of more than 2 (disease activity) was considered a treatment failure. At week 12, the proportion of patients with PGA more than 2 was five (33%) of 15 patients in the placebo group, two (13%) of 15 patients in the 150 mg AMG 714 group and none of 19 patients in the 300 mg AMG 714 group. The least square mean difference in PGA scores between 150 mg and placebo was –20% (95% CI –56 to 16) and not significant (p=0·39). The least square mean difference between 300 mg and placebo was –33% (–63 to –3) and had a nominal p value of 0·013.

In the per-protocol 2 population, with a very small sample size, the mean weekly total CeD GSRS, weekly total CeD PRO, and PGA scores did not differ significantly between the groups, and the BSFS data showed high variability (data not shown).

During the study period from the first dose of study drug administration to week 16 (6 weeks after the last dose), 60 (97%) of the 62 patients who received at least one dose of the study drug reported a total of 431 treatment-emergent adverse events (TEAEs), and 53 (85%) patients had at least one TEAE that was considered by the investigator to be related to the study drug (table 4). The incidence of overall or study drug-related TEAEs was similar between the AMG 714 and placebo groups.

No patients died or had a serious adverse event during the study. Two patients discontinued study treatment because of TEAEs. One of these patients had nausea, pharyngeal oedema, diarrhoea, arthralgia, and fatigue after two doses of 150 mg AMG 714; these events were considered to be possibly related to the study drug. One patient had temporal arteritis (Horton's arteritis) after five doses of 300 mg AMG 714; this event was considered by the investigator as not related to the study drug. Adverse events graded as severe occurred in one patient in the 300 mg AMG 714 group (arthralgia) and two patients in the placebo group (neck pain, abdominal pain, stomatitis, and fatigue).

As summarised in table 4, the organ system associated with the most TEAEs was the gastrointestinal system, which was probably related to the underlying disease and gluten challenge. Injection site reactions occurred more frequently in the AMG 714 150 mg group (eight [36%] patients) and the 300 mg group (11 [52%]) than in the placebo group (five [26%]). White blood cell counts increased modestly in one (5%) patient in the 150 mg group and three (14%) patients in the 300 mg group but in none of the patients in the placebo group. Other types of TEAEs did not show clear differences between the AMG 714 groups and the placebo group. Notably, infections and infestations occurred in 12 (55%) patients in the 150 mg AMG 714 group, ten (48%) in the 300 mg AMG 714 group, and nine (47%) in the placebo group.

No clinically significant trends of abnormalities were observed in clinical laboratory tests of haematology and urinalysis across the treatment groups. Most serum chemistry parameters showed no unexpected findings, but some increased liver function test results were seen. Abnormal liver function test results, including aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and total bilirubin, have been reported in about 10% of coeliac patients in Finland.[26] The liver

	150 mg AMG 714 group (n=22)	300 mg AMG 714 group (n=21)	Placebo group (n=19)
Patients with at least one TEAE	21 (95%)	20 (95%)	19 (100%)
Patients with at least one treatment-related* TEAE	19 (86%)	18 (86%)	16 (84%)
Patients who discontinued because of adverse events	1 (5%)	1 (5%)	0
Gastrointestinal disorders	17 (77%)	16 (76%)	13 (68%)
Abdominal distension	7 (32)	4 (19%)	6 (32%)
Abdominal pain	1 (5%)	3 (14%)	1 (5%)
Upper abdominal pain	1 (5%)	5 (24%)	4 (21%)
Diarrhoea	5 (23%)	8 (38%)	6 (32%)
Nausea	7 (32%)	4 (19%)	2 (11%)
General disorders and administration site conditions	11 (50%)	13 (62%)	11 (58%)
Fatigue	2 (9%)	5 (24%)	5 (26%)
Injection site reactions	8 (36%)	11 (52%)	5 (26%)
Infections and infestations	12 (55%)	10 (48%)	9 (47%)
Nasopharyngitis	5 (23%)	7 (33%)	7 (37%)
Investigations	4 (18%)	8 (38%)	5 (26%)
Hepatic enzyme increased	1 (5%)	1 (5%)	2 (11%)
White blood cell count increased	1 (5%)	3 (14%)	0
Musculoskeletal and connective tissue disorders	4 (18%)	8 (38%)	8 (42%)
Arthralgia	1 (5%)	4 (19%)	3 (16%)
Pain in extremity	1 (5%)	3 (14%)	3 (16%)
Nervous system disorders	6 (27%)	8 (38%)	9 (47%)
Headache	4 (18%)	7 (33%)	8 (42%)
Skin and subcutaneous tissue disorder	10 (45%)	2 (10%)	5 (26%)
Eczema	4 (18%)	0	1 (5%)
Pruritus	3 (14%)	1 (5%)	2 (11%)
Rash	4 (18%)	1 (5%)	0

TEAEs were reported and categorised by systems organ class and preferred term of the Medical Dictionary for Regulatory Activities. TEAE=treatment-emergent adverse event. *Related TEAEs included events considered by the investigators as definitely, probably, and possibly related to the study drug.

Table 4: **Summary of TEAEs during the treatment period that occurred in at least 10% patients in any group (intention-to-treat population)**

function test values increased in some study patients after the start of gluten challenge and later resolved after the end of gluten challenge. These changes were either similar across the three groups or were somewhat attenuated in the AMG 714 groups compared with the placebo group, but no statistical analysis was done (appendix p 6–7).

Patients who received AMG 714 might have had a modest increase in mean bodyweight, which in the context of coeliac disease is generally considered favourable (improved mucosal function). The mean change from baseline in bodyweight was 0·28 kg (SD 2·27) in the 150 mg AMG 714 group and 0·93 kg (1·89) in the 300 mg AMG 714 group, compared with 0·09 kg (2·03) in the placebo group. No statistical comparisons were made between groups, because bodyweight was not defined as an efficacy endpoint.

Six (14%) of the 43 patients who received at least one dose of AMG 714 tested positive for newly occurring antidrug antibodies at any post-treatment visit. At baseline, three patients in the AMG 714 groups and one in the placebo group had pre-existing antidrug antibodies. However, none of the samples were positive for neutralising antibodies.

Discussion

This was the first clinical trial to assess the efficacy and safety of an anti-IL-15 antibody, AMG 714 (also known as PRV-015) in coeliac disease. Patients whose symptoms had been well controlled on a gluten-free diet were given 2–4 g/day of gluten for 10 weeks to induce intestinal mucosal damage, inflammation, and symptoms in a controlled and reversible fashion. After 12 weeks of study treatment, the primary endpoint, change from baseline to week 12 in VHCD ratio, was not significantly different between placebo and AMG 714 150 mg or between placebo and AMG 714 300 mg. Mixed results were observed in other histological, clinical, and serological endpoints. Patients in the AMG 714 300 mg group showed better outcomes in the endpoints of change from baseline in intraepithelial lymphocyte density, CeD PRO score, and the proportion of patients who reported diarrhoea.

Although several experimental treatments for coeliac disease are in development, ranging from gluten-

destroying therapies to desensitising approaches,[27] no medications have been approved to date for marketing, and a gluten-free diet is the only effective treatment available to patients.

In this study, patients had a gluten challenge every day for 10 weeks during the study. Gluten challenge is a common approach to the assessment of potential treatments for coeliac disease, because it allows quite rapid analysis of the morphological and inflammatory responses to gluten in small bowel mucosa.[5,6] The dose of 2–4 g/day gluten challenge was considered substantial for patients who are on a gluten-free diet, because it is much higher than inadvertent dietary gluten intake, which has been estimated to be less than 1 g/day in 90% of patients on gluten-free diets.[28]

Methodologically, a major strength of this study was the administration of gluten-free cookies in a single-blind manner before initiating gluten challenge to understand symptoms due to nocebo effect (ie, potentially increased symptoms despite the absence of gluten challenge in the first 2 weeks of treatment), which allowed a comparison and understanding of the symptoms due to actual gluten consumption. Another strength of the study design was stratification based on age and sex, which reduced potential confounding factors. The use of stool and urine gluten tests to confirm gluten consumption has not previously been reported in drug or vaccine trials in coeliac disease. Additionally, the central preparation and reading of biopsy samples reduced the variability in histology and intraepithelial lymphocyte results.

The primary endpoint of protection from VHCD reduction showed no statistically significant difference between either dose of AMG 714 and placebo, indicating that treatment with AMG 714 by itself did not suppress gluten-induced mucosal damage and is probably not an appropriate treatment in the context of intentional consumption of gluten. However, several histological and clinical endpoints, including intraepithelial lymphocyte density, patient-reported and physician-assessed outcomes, and diarrhoea, showed amelioration of the effects of gluten challenge in the AMG 714 300 mg group, with smaller but consistent trends in the AMG 714 150 mg group. The totality of the results from the patients who had gluten challenge suggest that 300 mg AMG 714 given every 2 weeks might have measurable effects on inflammation and clinical symptoms caused by substantial gluten exposure.

Notably, the study findings confirm the purported role of IL-15 in the inflammatory processes in coeliac disease and suggest potential therapeutic benefits of anti-IL-15 treatment.[29] Although our results are preliminary and suggest a modest effect size, they support further research into potential use of AMG 714 as an adjunctive treatment to a gluten-free diet, particularly for the treatment of non-responsive coeliac disease. Patients with non-responsive coeliac disease remain symptomatic while on a gluten-free diet and present a significant unmet medical need. Mucosal abnormalities, increased intraepithelial lymphocytes, and recurrent symptoms persist in many patients despite adherence to a gluten-free diet.[11,30] Although most of the patients in this study were well controlled on a gluten-free diet at baseline (n=49) and could withstand gluten challenge, we did enrol and separately treat a small number of patients (n=11) who had substantial mucosal injury at baseline despite ongoing gluten-free diet—ie, patients with non-responsive coeliac disease. The individual efficacy results hinted at potential effects of AMG 714, but the small sample size and high variability precluded any firm conclusions. Future research of AMG 714 in patients with non-responsive coeliac disease will require a greater variety of doses, longer duration of treatment, and larger sample sizes to further elucidate the efficacy of AMG 714 in these patients.

AMG 714 was well tolerated in the study. No serious adverse events occurred, and most of the adverse events reported in the study were mild and consistent with the gluten challenge rather than the active treatment. The frequencies of adverse events, including infections, were similar between each AMG 714 dose group and the placebo group; the only exception was that injection site reactions occurred more frequently in AMG 714-treated patients. Additionally, no neutralising antibodies were detected in any sample. Although the long-term safety of AMG 714 has not been established, it has been shown that AMG 714 does not affect either natural killer cells or other white cell counts in humans.[23]

The study results should be interpreted with the limitations of a small and relatively short proof-of-principle study with only two doses tested and only 15–19 evaluable patients per treatment group. It should be noted that the VHCD ratio, although a validated diagnostic criterion for coeliac disease, has not been used in clinical trials to attain regulatory approval for a coeliac disease treatment. The effects of IL-15 inhibition on VHCD ratio and innate immunity have not been fully elucidated in coeliac disease research. Another potential limitation is that we could not identify or quantify possible non-compliance with a gluten-free diet in our per-protocol 1 population, given the gluten challenge. However, since the per-protocol 1 patients were screened for gluten-free diet compliance at study entry, we can reasonably expect that they adhered well to the protocol-specified instructions. Although gluten challenge was robust, variability in the exact amount of gluten delivered to each patient existed, and this variation was not included as a covariate in the analyses. The levels of anti-tTG and DGP IgA antibodies increased from baseline in all three groups in response to gluten and particularly in the 150 mg AMG 714 group, although the difference compared with placebo was not significant. This calls for attention in future studies but might also be a chance finding caused by variability in a small sample. Finally, the absence of validated assay methods to accurately quantify IL-15 concentrations in blood and tissues in the presence of an anti-IL-15 antibody

limited our ability to further illustrate the mechanisms of IL-15 in the pathophysiology of coeliac disease.

Despite the short study duration, this is, to the best of our knowledge, the longest published gluten-challenge trial completed to date with an experimental treatment for coeliac disease. Because IL-15 might be involved in gut inflammation and symptoms of coeliac disease, our findings support further research into the anti-IL-15 approach as possible adjunctive therapy to mitigate the harmful effect of dietary gluten contamination in patients struggling with continued symptoms despite adherence to a gluten-free diet. As gluten contamination has been shown to be common, such adjunct therapies could make an important contribution to the lifelong management of this disease. Indeed, AMG 714 (now also known as PRV-015) is currently in phase 2b development by Provention Bio and Amgen for the treatment of non-responsive coeliac disease in patients on gluten-free diets, with a greater variety of doses and durations of treatment. Additional research is needed to further identify the optimal dose regimen for anti-IL-15 treatment in coeliac disease and its long-term safety. Future research involving a larger number of biomarkers, such as cytokine profiles and validated IL-15 measurements in tissues, might be able to further clarify the effects of IL-15 blockade on the coeliac pathogenic cascade.

In this gluten challenge study of AMG 714, an anti-IL-15 monoclonal antibody, the primary endpoint of change in VHCD ratio from baseline after 12 weeks of treatment in patients with coeliac disease was not significantly different with the 150 mg or 300 mg AMG 714 dose compared with placebo. However, the 300 mg dose showed evidence of ameliorating the harmful effects of gluten as measured by intraepithelial lymphocyte density, patient-reported outcomes, and diarrhoea and was well tolerated. The findings support further research of AMG 714 in patients with coeliac disease that is not responsive to a gluten-free diet.

Contributors

MM, M-LL, and FL designed the study. MLL, MS, JT, AP, JL, JuK, O-PK, MP, LK, ZL, PV, JoK, JI, and MM conducted the study procedures and collected and analysed data. FL, JRP, JI, and MM analysed and interpreted the data.

Declaration of interests

LK reports personal fees for lectures from Finnish Coeliac Society outside the submitted work. FL was the chief executive and medical officer of Celimmune during the conduct of the study and was a consultant for Amgen; is chief scientific officer of and owns stocks in Provention Bio, which is in a partnership with Amgen to develop AMG 714/PRV-015; he owned stock in Biomedal during the conduct of the study and has a pending patent for methods and compositions for the treatment of coeliac disease, non-coeliac gluten sensitivity, and refractory coeliac disease. ZL was an employee of Clinical Research Services Turku (CRST), which was one of the study sites, during the conduct of the study. MM reports personal fees from Celimmune during the conduct of the study; and is an advisor or consultant for ImmusanT, ImmunogenX, Innovate Biopharmaceuticals, Dr Falk Pharma, Actobio Therapeutics, Jilab, and Ukko, outside the submitted work. MS is a shareholder, employee, and board member of CRST, which was one of the study sites; and was chair of the ethics committee of Southwest Finland Hospital District through the end of 2018, outside the submitted work. JRP is an employee of Amgen. JT is an employee of FinnMedi, which was one of the study sites, during the conduct of the study. JI owns stock in Jilab. AP was a contractor to Jilab. All other authors declare no competing interests.

Data sharing

There is a plan to share data. This might include de-identified individual patient data for variables necessary to address the specific research question in an approved data-sharing request as well as related data dictionaries, study protocol, statistical analysis plan, informed consent form, or clinical study report. Data sharing requests relating to data in this manuscript will be considered after the publication date and after this product and indication (or other new use) have been granted marketing authorisation in both the USA and Europe or after clinical development discontinues and the data will not be submitted to regulatory authorities. No end date exists for eligibility to submit a data sharing request for these data. Qualified researchers may submit a request containing the research objectives, the Amgen product(s) and Amgen study or studies in scope, endpoints or outcomes of interest, statistical analysis plan, data requirements, publication plan, and qualifications of the researcher(s). In general, Amgen does not grant external requests for individual patient data for the purpose of re-evaluating safety and efficacy issues already addressed in the product labelling. A committee of internal advisors reviews requests. If not approved, a data sharing independent review panel will arbitrate and make the final decision. Upon approval, information necessary to address the research question will be provided under the terms of a data sharing agreement. This might include anonymised individual patient data or available supporting documents containing fragments of analysis code where provided in analysis specifications. Further details are available on the Amgen website.

For the **Amgen clinical trial data sharing request policy** see http://www.amgen.com/datasharing

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