Efficacy and safety of gluten peptide-based antigen-specific immunotherapy (Nexvax2) in adults with coeliac disease after bolus exposure to gluten: a phase 2 randomised, double-blind, placebo-controlled study

Short Title: Efficacy and safety of Nexvax2 in coeliac disease

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Author declaration The authors made the decision to submit the manuscript for publication and vouch for the accuracy of data and analyses and for the fidelity of this report to the trial protocol. All authors contributed to manuscript revision and approved the text, tables and figures. RPA had full access to all data in the study and had final responsibility for the decision to submit for publication.

The NIH did not fund the study, and none the authors received funding from NIH.

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Research in context

Evidence before this study Treatment of coeliac disease by gluten-free diet alone is partially effective, but most patients have persistent intestinal injury and are susceptible to acute gastrointestinal reactions associated with systemic cytokine release after gluten. Ameliorating gluten-induced symptoms and intestinal injury are the goals of therapeutic development in coeliac disease. The search term "celiac disease" in Clinicaltrials.gov reveals 29 separate therapeutic candidates have been assessed from 2006 to 30th March 2022 with only 10 commercially backed agents having completed phase 2 trials with one that reached phase 3 but was recently discontinued after an interim analysis. The 2 – 13 weeks duration of gluten challenge in trials found in Clinicaltrials.gov assessing histology or symptoms is worrying because of selection bias favouring patients who can "tolerate" gluten without disabling symptoms. Coeliac disease is an antigen (gluten)-driven disease with an exceptionally well characterised CD4+ T cell response making it uniquely suited for antigen-specific immunotherapy. There is, however, no approved antigen-specific immunotherapy for any non-allergic immune disease, and no approved therapeutic for coeliac disease. Nexvax2 is the most advanced of three antigen-specific immunotherapy in clinical development for coeliac disease. Gluten-specific CD4+ T cells are suppressed by Nexvax2 and are responsible for acute symptoms in coeliac disease, but convincing evidence that Nexvax2 or any other investigational product modifies gluten-induced symptoms in coeliac disease has been lacking.

Added value of this study The RESET CeD Study demonstrated the utility of single bolus vital gluten challenge combined with serum interleukin-2 measurement is an effective new tool for screening and efficacy endpoints during clinical development of therapies for coeliac disease. Despite ex vivo gluten peptide-specific T cell responses being substantially reduced by Nexvax2 gluten-peptide antigen-based immunotherapy, interim analysis prompted discontinuation of the RESET CeD Study because acute symptoms and systemic interleukin-2 release after masked bolus 10-gram vital wheat gluten challenge in patients was unaffected. In contrast, intestinal histology showed statistically significant improvement after the 18-week treatment period with Nexvax2.

Implications of all the available evidence Amelioration of acute symptoms induced by heavy gluten challenge appears to be among the hardest endpoints to achieve in therapeutic trials of investigational products for coeliac disease. The RESET CeD Study points to the importance of designing coeliac disease trials to address the intended purpose of experimental therapeutics whether they are to control low gluten exposure and serve as a supplement to dietary therapy or to powerfully suppress gluten immunity allowing an unrestricted diet.

Summary

Background. Gluten-free diet is insufficient to treat coeliac disease because intestinal injury persists and acute reactions with cytokine release follow gluten exposure. Nexvax2 is a specific immunotherapy utilizing immunodominant peptides recognized by gluten-specific CD4+ T cells. We aimed to assess the effects of Nexvax2 on gluten-induced symptoms and immune activation using a simplified study design shortening gluten challenge to one exposure.

Methods. This was a 41-site randomised, double-blind, placebo-controlled, phase 2 study. Coeliac disease participants (were on gluten-free diet, HLA-DQ2·5+, 18–70 years, and at screening unmasked 10g vital gluten challenge worsened symptoms. Primary analysis group was HLA-DQ2.5-non-homozygous participants (n = 154, 77% females) randomly assigned (1:1) twice weekly subcutaneous Nexvax2 or saline escalating from 1 to 750μg over 5-weeks then 900μg for 11 weeks. The primary endpoint was change from pre-treatment baseline in CeD PRO "Total gastrointestinal domain" on the day of masked bolus 10g vital gluten challenge given in week-14. Secondary endpoints included individual digestive symptoms and change in serum interleukin-2 at 4 hours on the day of bolus gluten challenge. Duodenal histology and drug exposure were assessed (ClinicalTrials.gov NCT03644069).

Findings 179 of 383 volunteers were randomized 21 September 2018 to 24 April 2019. The study was discontinued after planned interim analysis in 66 non-homozygous subjects. Once the decision to discontinue the study was made on 25 June 2019, the investigators were unblinded to study data. The current report describes an unblinded post hoc analysis combining data from 67 non-homozygous subjects assessed in the planned interim analysis as well as two additional subjects who completed the first masked gluten food challenge up to 3 June, 2019. None of the primary and secondary endpoints associated with bolus gluten challenge were achieved (primary endpoint P = 0.43). Nexvax2 and placebo adverse events and 4h post-dose interleukin-2 were similar. Serious adverse events were reported in one (1%) of Nexvax2-treated subjects during gluten challenge (left-sided mid-back muscle strain with imaging suggestive of partial left kidney infarction) and in six (7%) of placebo-treated subjects (exacerbation of asthma, forehead abscess, conjunctivitis, folliculitis, and appendicitis). The most frequent adverse events affecting Nexvax2 and placebo-treated subjects were nausea (48% versus 34%), diarrhoea (35% versus 29%), abdominal pain (34% versus 32%), headache (35% versus 23%) and fatigue (26% versus 36%). Median 45-minute post-dose Nexvax2 peptide concentrations were 6.4 – 9.2 ng/ml during maintenance.

Interpretation Nexvax2 did not reduce acute gluten-induced symptoms or immune activation, but duodenal histology improved. Masked bolus vital gluten challenge provides an alternative to extended gluten challenge in efficacy studies for coeliac disease.

Funding The study was funded by ImmusanT Inc., but played no role in writing the paper. ImmusanT was involved in all aspects of the study except writing the manuscript. The authors were not paid to write the manuscript. The authors were not precluded from accessing data in the study, and they accept responsibility to submit for publication.

Introduction

Coeliac disease (CeD) is due to a proinflammatory CD4+ T cell-driven adaptive immune response directed against partially deamidated gluten peptides.[1] Strict, life-long gluten-free diet (GFD) is currently the only option for CeD. Many drug candidates are in development for CeD but none have successfully completed phase 3 and few have progressed to large phase 2 studies.[2-4] Substantial debate continues regarding efficacy endpoints and recently, the utility of elevation in serum interleukin-2 (IL-2) linked to symptoms within hours after acute gluten exposure has been proposed as a possible alternative efficacy endpoint to intestinal histology after prolonged gluten challenge.[5]

Unexpectedly, requiring immune and symptom efficacy measures for the clinical development of gluten-specific immunotherapy prompted patient studies that revealed unrecognised aspects of CeD pathophysiology including systemic cytokine release after gluten and the prominent role IL-2.[6,7] Here we report the phase 2b RESET CeD Study of the first potential antigen-specific immunotherapy (ASIT) assessed for CeD, Nexvax2, that sought to modify gluten-induced symptoms and systemic IL-2 release. The novel study design is the first to exploit vital wheat gluten food challenge shortened to a single bolus exposure. Gastrointestinal symptoms driven by bolus vital wheat gluten challenge allowed provided a measure of disease severity to assess eligibility during screening and for the primary and secondary endpoints.[8]

ASIT is well established for laboratory models of autoimmune disease, and is an important emerging class of potential therapies for coeliac disease.[9] ASIT aims to restore "immune tolerance", which is classically defined as "a state of indifference or non-reactivity towards a substance that would normally be expected to excite an immunological response".[10] ASIT holds unique appeal as a therapeutic strategy because it promises to selectively suppress or delete disease-causing antigen-specific CD4+ T cells.[9] ASIT began with whole protein desensitisation to suppress or even cure specific allergies.[11] For CeD, the gluten proteins are unsuitable as an immunotherapy because they are insoluble. Instead, Nexvax2 is a "peptide-based immunotherapy",[11] and was developed using soluble synthetic peptides corresponding to "dominant" epitopes for gluten-specific CD4+ T-cells in the 90% of CeD patients positive for HLA-DQ2.5.[12] The formulation of Nexvax2 is the simplest of the ASIT's under development for CeD and does not include an adjuvant or specialized delivery system. Nexvax2 is administered once or twice weekly and given by intradermal or subcutaneous injection providing systemic exposure for up to six hours.[8,13] Nexvax2 at dose levels of 60 μg or higher causes an acute first-dose gastrointestinal reaction associated with transiently elevated blood levels of IL-2, IL-10 and several chemokines,[6,8,14] but this is prevented by initial up-dosing allowing patients to tolerate doses of 900 μg without acute symptoms or immune activation.[13,15]

Prompted by the initial phase1/2a Nexvax2 study findings, the effects of single bolus food challenge with vital gluten were shown to closely resemble the first dose reaction to Nexvax2.[6] This observation suggested symptom and immune endpoints in clinical trials of investigational therapies for CeD could be assessed without burdening volunteers with conventional gluten challenges over many weeks necessary to show histological relapse in duodenal biopsies.[16] In fact, even histology endpoints might be achievable without gluten challenge by assessing healing on treatment; high-performance quantitative histomorphometry unexpectedly revealed persistent villous atrophy and crypt hyperplasia unrecognised by conventional histology at baseline in almost 60% of CeD patients "well-controlled" on GFD enrolling in Nexvax2 trials.[17]

The aim of this report was to describe the study design and main outcomes of the RESET CeD Study after an interim analysis caused its discontinuation. While the primary endpoint was not met, the RESET CeD Study provides important insights for development of ASITs, and also informs the design of future CeD therapy trials. Because of the pressing need to improve the design of efficacy studies supporting regulatory approval of therapies for coeliac disease, several aspects of the discontinued RESET CeD Study have already been reported. These include the clinical and IL-2 responses to unmasked gluten challenge at screening,[18] masked gluten challenge during the treatment period in patients receiving placebo,[19] and ultra-sensitive whole blood IL-2 release test to monitor T-cell responses to Nexvax2 and gluten peptides without the requirement for prior gluten challenge.[20]

Materials and Methods

Study design and participants

Figure S1 shows the study design. The RESET CeD Study (ClinicalStudys.gov Identifier: NCT03644069) was a randomised, double-blind, placebo-controlled study conducted at 41 clinical sites listed in table S1. Sites obtained independent institutional review board or ethics committee approval to conduct the study (table S2), and all enrolled patients freely gave informed consent prior to undergoing any study-related procedures. The study was conducted in accordance with the ethical principles in the Declaration of Helsinki, and consistent with Good Clinical Practice (International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; Harmonised Tripartite Guideline E6 [R1]), as well as with all applicable national and local regulatory requirement(s). ICON plc (Leopardstown, Dublin 18, Ireland) managed the study.

The primary objective of the study was to evaluate the efficacy of Nexvax2 compared with placebo in reducing gastrointestinal symptoms in CeD patients on a GFD after masked food challenge containing gluten. Secondary objectives included evaluating the effect of Nexvax2 on T-cell activation after gluten challenge, and safety and tolerability of Nexvax2. Exploratory objectives included Nexvax2's effect on duodenal histology for safety, and to evaluate drug exposure.

Eligible patients were aged 18 to 70 years with CeD diagnosed on the basis of duodenal villous atrophy and abnormal serology, and had excluded gluten for at least one year. Based on screening tests patients were included only if they were positive for the HLA DQ2·5 genotype (*HLA-DQA1*05* and *HLA-DQB1*02* alleles) and reported a worsening in the patient-reported 10-point Global Symptom Survey (GloSS) score of at least 3 within 6-hours after screening (unmasked) 10g bolus vital gluten challenge, or onset of a gastrointestinal adverse event graded by site staff as at least moderate severity (CTCAE, Version 4.03 Grade 2) before midnight on the day of screening gluten challenge. Full eligibility criteria are listed in table S3.

Randomisation and masking

Eligible patients were randomised to one of four treatment groups by a central integrated web response system in a blinded fashion according to HLA-DQ2.5 genotype. HLA-DQ2.5 non-homozygous patients were randomised to Nexvax2 or placebo in a 1:1 ratio. A subset of the non-homozygous patients who consented to having endoscopy with duodenal biopsy were enrolled in a biopsy cohort. The exploratory population of HLA-DQ2.5 homozygous patients were randomised to Nexvax2 or placebo in a 2:1 ratio.

Details of the study drug, administration and masking are provided in Appendix. Study patients, study site personnel and data managers remained blinded to study drug treatment assignment.

Procedures

A complete list of screening assessments is in the Schedule of Assessments shown in table S4 in the Appendix. At screening, HLA-DQ genetic test was performed (UCLA Immunogenetics Center, Los Angeles, CA). At least six weeks before the treatment period, patients consumed a "screening food challenge". During a six-hour observation period, patients used a handheld electronic diary to record their overall severity of gastrointestinal symptoms on a 0 to 10 scale (GloSS) each hour. Serum IL-2 concentrations were measured immediately before gluten challenge and at 2, 4 and 6 hours as described elsewhere.[18] A subset of non-homozygote patients in the biopsy cohort had baseline second part duodenal histology assessed by quantitative histology (JiLab Inc., Tampere, Finland) according to methods described elsewhere.[17]

Dosing with blinded Nexvax2 or placebo occurred twice a week during the dose escalation phase when dose levels of 1, 3, 9, 30, 60, 90, 150, 300, 450, 600, and 750 μg were delivered subcutaneously by site staff. Patients were observed for four hours after the first dose and for at least 30 minutes after later doses. A dose level could be administered up to three times if a patient experienced study drug-related emergent gastrointestinal symptoms on the day of dose administration that was at least Grade 2 according to the CTCAE, Version 4.03.

During the 11-week maintenance phase patients self-administered 900 μg of Nexvax2 or placebo twice weekly. Patients consumed three on-site, double-blind, low FODMAP, masked bolus food challenges at two-week intervals beginning in week 12 of the treatment period (Rutgers Food Innovation Center; Bridgeton, NJ, USA).[19] The first food challenge was gluten-free "sham" and the second was vital wheat gluten. Patients were randomised 2:1 for the third food challenge to receive gluten or sham. There was a four-week post-treatment period. Biopsies were performed in the first week post-treatment.

An electronic handheld diary was used to collect patient reported outcomes between 6:00 pm and midnight. Each day patients completed the 9-point CeD patient reported outcome ("CeD PRO"),[2] the Bristol Stool Form Scale and a Patient Global Assessment of bowel function daily. At specific times throughout the study, patients also completed the Impact of Celiac Disease Symptoms Questionnaire, the Patient Global Assessment of symptom severity and the Short Form Health Survey Version 2. Serum IL-2 was assessed before and 4-hours after food challenges, the first dose, the first maintenance dose, and also at end-of-treatment. The lower level of quantitation for IL-2 was <0.5 pg/ml, and for purposes of calculating net change baseline level was considered 0.5 pg/ml unless higher levels were recorded.[18] Safety assessments during the treatment period included vital signs, safety labs, and adverse event monitoring. Adverse events were recorded at each visit and graded by site staff according to CTCAE v4.03. Additional laboratory assessments included CeD specific-serology and pharmacokinetics.

Outcomes

The primary endpoint was the difference between CeD PRO "Total gastrointestinal (GI) domain" score averaged over the 14-day interval immediately prior to the treatment period and the day of the first masked gluten food challenge (MFC2) at Visit 34 and was analysed by ANCOVA at the 1-sided 5% significance level. The Total GI Domain score was the mean of the "Abdominal Symptoms" score, the "Diarrhea and Loose Stools" score, and the "Nausea" item score; the

Abdominal Symptoms score was the mean of item scores for abdominal cramping, abdominal pain, bloating, and gas; and the "Diarrhea and Loose Stools score" was the mean of daily item scores for "diarrhea" and "loose stools". Change in the log-transformed IL-2 serum concentration from within 30 minutes before to 4 hours after the first masked gluten food challenge (MFC2) analysed by ANCOVA was a secondary endpoint. Differences between baseline and day of MFC2 for individual symptoms and symptom domains were secondary outcomes assessed by CeD PRO analysed by ANCOVA (Abdominal Symptoms score, Diarrhea and Loose Stools score, and each of the seven individual GI item scores in the CeD PRO). The other secondary objective was to evaluate the safety and tolerability of Nexvax2 as assessed by standard clinical and laboratory measures including treatment emergent adverse events (TEAEs), vital sign measurements, and clinical laboratory information. An exploratory objective was to evaluate the effects of Nexvax2 compared with placebo on duodenal histology. Duodenal histology was analyzed as a safety endpoint and as a post hoc exploratory endpoint comparing post-treatment to baseline including measurements of villous height (VH), crypt depth (CrD), the sum of paired measurements of VH and CrD, and the villous height to crypt depth ratio (VH:CrD) as well as the density of intra-epithelial CD3+ lymphocytes. An exploratory endpoint was to evaluate the relationship between the pharmacokinetics (PK) of Nexvax2 after the first and penultimate maintenance dose administrations of Nexvax2 (900 μg) assessed in pre-dose and 45-minute post-dose blood samples.

Statistical Analyses

Analyses were conducted for the primary cohort of subjects heterozygous for *HLA-DQA1*05* and/or *HLA-DQB1*02* alleles (HLA-DQ2·5 "non-homozygotes") separately from those for subjects homozygous for both *HLA-DQA1*05* and *HLA-DQB1*02* alleles (HLA-DQ2·5 "homozygotes") because an earlier study suggested HLA-DQA and DQB gene-dose may influence Nexvax2 tolerability.[6,8] "Homozygotes" were enrolled into a separate cohort to assess exploratory efficacy endpoints. Analyses of homozygous patients were exploratory only.

The Intent-to-treat (ITT) Population consisted of all randomized patients who received at least 1 dose of IP. The Safety Population comprised all patients who received IP. The Per-protocol Population (PP population) consisted of all patients who completed all 3 MFCs, received all planned IP administrations, and had no important protocol deviations. The Biopsy-evaluable Population (HLA-DQ2.5 non-homozygote endoscopy subset) consisted of all patients who had duodenal biopsies collected and were evaluable for quantitative histology in both the screening period and 7±2 days after EOT. The Screening Food Challenge Population consisted of all patients who received gluten during the SFC, including patients who were not randomized to the study. Primary and secondary efficacy endpoints were assessed in the ITT Population participants who were HLA-DQ2·5 "non-homozygotes".

The estimated sample size of 128 yielded approximately 80% power for the primary endpoint comparison of the Nexvax2 and placebo arms in the HLA-DQ2.5 non-homozygote cohort. This estimate was based on a treatment effect size of 0.5, a 2-sample t-test at the 1-sided 5% significance level, and a 20% drop-out rate. It was expected that approximately 292 patients would be screened in order to randomise 64 HLA-DQ2.5 non-homozygous patients to both the Nexvax2 arm (A) and placebo arm (B). Initially, 18 HLA-DQ2.5 homozygous patients randomised in a 2:1 ratio to receive Nexvax2 (Arm C) or placebo (Arm D) was deemed adequate

to address the key exploratory objective of assessing safety and tolerability in HLA-DQ2.5 homozygous patients.

Summary statistics are displayed as either mean (standard deviation; SD) or median (interquartile range; IQR) for continuous data as appropriate, and as frequency (percentage) for categorical data. Study outcomes were analysed as predefined in the statistical analysis plan, with continuous outcomes analysed using analysis of covariance models. In each model, treatment group (Nexvax2/placebo) and the baseline value of the outcome variable were included as fixed effects in the model. Effect estimates were reported as mean difference (MD) and 95% confidence interval (95% CI). Analysis was undertaken using Stata statistical software v14 (StataCorp, College Station, TX, USA).

Role of the funding source

The study funder had roles in study design; data collection, analysis, and interpretation; and the writing of this Article. GG, LJW, and RPA had full access to all the data in the study. The corresponding author had final responsibility for the decision to submit for publication.

Results

Altogether, 383 patients were screened from 21 September 2018 to 24 April 2019, and 179 patients were randomised to treatment with Nexvax2 or placebo. Due to rapid screening by several sites and many patients meeting eligibility before screening closed, the study was over-enrolled and ultimately Arms A and B included 76 and 78 subjects, respectively, and Arms C and D included 16 and 8 subjects. The last patient last visit was 26 July, 2019. As shown in figure 1, 73 of the 204 excluded patients were discontinued prior to the screening unmasked gluten food challenge. Insufficient deterioration in symptoms after screening gluten challenge resulted in exclusion of 39 patients; patient responses to screening gluten challenge are reported in detail elsewhere.[18] Altogether, 154 non-homozygous patients were assigned to the primary endpoint cohort and their treatment assignment was 76 Nexvax2: 78 placebo. Demographics of all non-homozygotes randomised (intention-to-treat population) are shown in table 1. In addition, 16 homozygous patients received Nexvax2 and 8 received placebo in an exploratory cohort.

A planned interim efficacy analysis addressed only the primary (symptom) efficacy endpoint and safety data, and was performed on the subset of 66 non-homozygote subjects who had completed the first two masked food challenges and data collected up to and including 31 May, 2019. Although the interim analysis had initially not been intended as a go-no-go decision-point, a decision was taken on 25 June 2019 to discontinue the study and the investigators were unblinded to study data. The final data provided to the sponsor was on 23 August, 2019 and included additional safety data, IL-2 data relating to the secondary efficacy endpoint, pharmacokinetics (first and last maintenance doses), and histology. The current report describes a subsequent unblinded post hoc analysis of data generated for the planned interim analysis as well as additional CeD PRO data from two non-homozygous subjects who completed the first masked gluten food challenge up to 3 June, 2019.

The primary endpoint was assessed in 33 of the 76 Nexvax2-treated and 34 of the 78 placebo-treated patients in the ITT population. Demographics of the subgroups of patients treated with

Nexvax2 or placebo and assessed for the primary endpoint were representative of those in the ITT population (table 1), and their symptoms at baseline were also similar (table S5).

The primary endpoint was not met when assessed with all available data (table 2), the mean (SD) change in Total Gastrointestinal Score between baseline and the day of first gluten challenge was 2.86 (2.28) in 33 Nexvax2-treated patients compared to 2.63 (2.07) in 34 Nexvax2 treated patients (MD = 0.41; 95%CI: -0.62, 1.44; P = 0.43).

Secondary symptom endpoints were not met when assessed with all available data (table 2). In fact, the mean change in Abdominal Domain and the Bloating individual symptom score were worse in the Nexvax2 group than in placebo-treated patients.

Table S6 shows CeD PRO data for non-homozygous subjects in the ITT population at baseline, and for those with data available after sham and first masked gluten food challenge. As previously reported, masked gluten food challenge (MFC2) worsened symptoms compared to sham (MFC1). [19] For those subjects included in the primary endpoint analysis, Total Gastrointestinal Score was similar for Nexvax2 and placebo on the first day of treatment when Nexvax2 1 μg was administered (Visit 5) and also five weeks later when the Nexvax2 900 μg maintenance dose was first administered (Visit 16).

The secondary endpoint comparing the difference between Nexvax2 and placebo groups in change of log-transformed IL-2 from baseline was not met (MD = -0.4, 95% CI: -1.4 – 0.6, P = 0.41). Figure 2a and 2d show median levels of serum IL-2 four hours after the masked gluten food challenge (MFC2) at Visit 34 were similar for the 31 HLA-DQ2.5 non-homozygous Nexvax2-treated subjects (median 9.3 pg/ml, IQR: 0.7 – 22) and 32 treated with placebo with available data (7.8, 1.7 - 38). Serum IL-2 concentration was consistently <0.5 pg/ml before and after masked sham food challenge at Visit 28, and also before masked gluten food challenge (MFC2) at Visit 34. Figure 2b and 3e show change in serum IL-2 after gluten challenge at screening and Visit 34 were significantly correlated for individuals in both the Nexvax2 and placebo groups. Figure 2c and 3f show change in total gastrointestinal symptom score was correlated with change in serum IL-2 after gluten challenge at Visit 34.

During the study period from the first dose of study drug administration to week 21 (4 weeks after the last dose), 68 (89%) of the 76 HLA-DQ2.5 non-homozygous patients who received at least one dose of Nexvax2 reported a total of 793 treatment-emergent adverse events (TEAEs), and 60 (79%) patients had at least one TEAE for a total of 463 TEAEs that was considered by the investigator to be possibly or probably related to the study drug (table 4). The incidence of overall or study drug-related TEAEs was similar between the Nexvax2 and placebo non-homozygous patient groups with 72 (92%) of the 78 placebo-treated patients experiencing 821 TEAEs, and 59 (76%) experiencing at least one TEAE for a total of 477 individual TEAEs that was considered by the investigator to be related or possibly or probably related to the study drug. Two patients discontinued study treatment because of TEAEs. One of these patients was receiving placebo and withdrew after an “allergic reaction” on the day of the first masked gluten food challenge (MFC2) graded moderate severity. The other patient was receiving Nexvax2 and withdrew after the first masked gluten challenge (MFC2) when he developed vomiting, diarrhoea, and left-sided mid-back muscle strain that led to investigation with imaging

suggestive of partial left kidney infarction, which was graded as a serious adverse event (SAE). There were six other SAEs, with five in participants receiving placebo and included exacerbation of asthma, forehead abscess, conjunctivitis, folliculitis, and appendicitis. One participant receiving Nexvax2 developed a pulmonary embolism one week after end of treatment that was also graded a SAE. As summarised in table 4, the organ system associated with the most patients having TEAEs related to study drug was the gastrointestinal system, which was probably related to the underlying disease and gluten challenges during the maintenance dosing period. Indeed, the TEAEs that patients reported most often are those that are frequently linked to gluten in masked and unmasked food challenge in CeD,[18,19,21] or after initial dosing of treated CeD patients with Nexvax2.[8] TEAEs did not show clear differences between the Nexvax2 groups and the placebo groups overall or during the updosing period, which supported the effectiveness of gradual dose escalation of Nexvax2 in overcoming the acute symptoms associated with Nexvax2 doses above 30 μg. Similarly, there were no apparent differences in HLA-DQ2.5 homozygotes dosed with Nexvax2 or placebo. No clinically significant trends of abnormalities were observed in clinical laboratory tests of haematology and urinalysis across the treatment groups.

Nexvax2 peptides reach maximal levels at 45 minutes and have half-lives of two-hours after subcutaneous administration in CeD patients on GFD.[13] After Visit 16 (first maintenance dose at 900 μg) and after the last dose at end-of-treatment (Visit 42) plasma levels of Nexvax2 peptides were available for 28 of the Nexvax2-treated patients (HLA-DQ2.5 non-homozygotes and homozygotes) and 26 of the placebo-treated patients. As expected, none of the Nexvax2 peptides were detected in plasma before administration or after placebo. Plasma levels for each of the Nexvax2 peptides were above lower levels of quantitation (0.5 ng/ml, 0.3 nM) after dosing at Visit 16 (NPL001 median 9.2 ng/ml, range 1.6 – 15.1; NPL002 median 8.8 ng/ml, 1.9 – 24.1; and NPL003: 7.3 ng/ml, 1.6 – 11.8) and at Visit 42 (NPL001 median 8.0 ng/ml, range 1.9 – 17.2 ng/ml; NPL002 median 7.9 ng/ml, 0.6 – 18.8; and NPL003: 6.4 ng/ml, 1.9 – 15.5). These peptide levels were consistent with previously reported maximal peptide concentrations following subcutaneous administration of Nexvax2 900 μg,[13] and confirmed systemic bioavailability of Nexvax2 peptides during the dosing period.

Quantitative duodenal histology at baseline was similar for the Nexvax2 group (n = 13) and placebo group (n = 14) with median villus height to crypt depth ratio (VH:CrD) being 1.67 and 1.66, respectively (table 4). Table 4 shows VH:CrD after the end of the treatment period had improved significantly in the 13 Nexvax2-treated patients compared to the 14 placebo-treated patients (1.80 versus 1.57, respectively; MD = 0.22, 95%CI: 0.06, 0.39; P=0.01 by ANCOVA). Improvement in VH:CrD in Nexvax2-treated patients was due to significantly increased villus height (table 4). According to 0.4 or more being a clinically significant change in VH:CrD,[22] the Nexvax2 group included two patients whose VH:CrD increased 0.4 or more and none reduced by 0.4 or more whereas the placebo group included two who reduced by 0.4 or more and none increased 0.4 or more. Change in intraepithelial lymphocyte density was not significantly different between Nexvax2 and placebo-treated patients.

Discussion

Analyses of all available data from 33 HLA-DQ2.5 non-homozygous patients receiving Nexvax2 and 37 receiving placebo indicated Nexvax2 was safe and well tolerated, and Nexvax2 exposure

was confirmed and did not change over the treatment period in the RESET CeD Study. Nexvax2 treatment did not modify digestive symptoms during the day after participants consumed a masked 10-gram bolus of vital wheat gluten, about half the 13 grams of gluten typically consumed daily by an adult.[23] The secondary endpoints of serum IL-2 elevation at four hours after the same bolus gluten challenge, and individual digestive symptoms also showed no benefit of Nexvax2 treatment.

In contrast, quantitative histomorphometry on duodenal biopsies showed Nexvax2 was associated with statistically significant increases in villus height to crypt depth ratio with two of 13 Nexvax2-treated subjects increased by more than 0.4. Furthermore, patients were asymptomatic and did not elevate serum IL-2 after receiving maintenance 900 μg doses of Nexvax2, which is more than 10-times the minimum dose that causes acute gastrointestinal reactions when administered on a single occasion in Nexvax2-naïve CeD patients.[8] In addition, we have reported that Nexvax2 treatment (in a subgroup of patients in the RESET CeD Study) reduced fresh blood IL-2 release by 70 - 90% and abolished interferon-γ secretion stimulated by Nexvax2 gluten peptides as well as by gluten peptides not included in Nexvax2.[20]

Collectively, combining the findings of previous clinical trials of Nexvax2 with those from the RESET CeD Study, Nexvax2 treatment appears to induce clinical and immunological hypo-responsiveness to itself,[8] and suppresses Nexvax2 gluten epitope-specific CD4+ T-cell immunity.[20] But this level of "immune tolerance" to gluten peptides in Nexvax2 was inadequate for sudden exposure to a large amount of gluten after overnight fasting. Hence, future product claims of restoring immune tolerance to gluten may need to be nuanced by assessments of clinical and immune responsiveness across a range of gluten doses encountered by CeD patients whether they are carefully avoiding gluten or are consuming an unrestricted diet.

Dosage and dose intervals of Nexvax2 in the RESET CeD Study were guided by clinical symptoms, plasma IL-2 levels after administering Nexvax2 and after 10-gram bolus gluten challenge, and also by monitoring gluten-specific CD4+ T cells following 3-day gluten challenge in prior Nexvax2 trials.[8,15] The comparable levels of plasma IL-2 and gastrointestinal symptoms after one 150 μg dose of Nexvax2 versus ten grams vital wheat gluten suggested that maintenance Nexvax2 dosing at 900 μg would be sufficient to reduce clinical effects of consuming 10 grams vital wheat gluten. Potentially, Nexvax2 could have protected against lower doses of gluten, or a higher maintenance dose of Nexvax2 dose could have been more effective. Indeed, gluten peptide-stimulated whole blood cytokine release suggested that gluten-specific CD4+ T cells in Nexvax2-treated patients were capable of antigen-induced cytokine secretion.[24]

Gluten-induced symptoms and intestinal histology have been emphasized as the efficacy endpoints for CeD trials.[25] Uncertainty over what symptoms gluten causes, at what dose, and in what format were barriers to undertaking a symptom-based efficacy study in CeD. Even though patient reported outcome instruments have been developed in accordance with regulatory guidelines, they were based on retrospective recall of symptoms and had not included gluten challenge studies or consideration of the effects of FODMAPs in wheat flour causing irritable bowel syndrome.[26] The Nexvax2 programme revealed nausea and vomiting rather than diarrhoea are the cardinal symptoms caused by recent gluten exposure,[18,19,21] and showed that systemic cytokine release accompanies acute gluten reactions specifically in CeD.[6,14,27] In a pilot study, we had demonstrated that 10-gram vital gluten stimulates symptoms that were generally "moderate" and correlated with a relevant biomarker – IL-2.[21] As such the 10-gram (low

FODMAP) vital wheat gluten format employed in the RESET CeD Study was the first reported gluten food challenge to be linked to an immunologically relevant and temporally related biomarker, and also induced reproducible, measurable but tolerable symptoms in a double-blind sham-controlled format.[19]

Blood IL-2 level is now positioned as a marker of gluten-specific CD4+ T-cell activation *in vivo* and *ex vivo*, and potentially may serve as a surrogate efficacy endpoint because it correlates in timing and with severity of symptoms caused by gluten in CeD patients on GFD.[6,14,21,24] Others have since confirmed *in vivo* IL-2 release four hours after gluten, which appears to be a more sensitive marker of gluten exposure than duodenal histology after consuming gluten for two weeks.[28]

Discontinuing the RESET CeD Study was clearly a weakness in this report as endpoint data was unavailable for half the randomized patients, and exploratory endpoints were not assessed. There were, however, several important strengths in the study design. As we have already reported,[19] the bolus gluten challenge was matched by a sham also low in FODMAP content providing clear evidence of gluten-specific symptoms. In contrast, a recent study confirmed elevations of serum IL-2 after bolus gluten challenge, but the sham and gluten (bread from flour) challenge articles were not assessed for FODMAP content and symptoms were not specific for gluten.[29] A further strength was to stratify "severity" of coeliac disease during screening using an unmasked format of the 10-gram vital gluten challenge, which allowed for randomization of patients more symptomatic after gluten. As we have already reported,[18] peak serum IL-2 levels within six hours after gluten ranged from less than 0.5 to over 1000 pg/ml and correlated with overall gastrointestinal symptom severity. Although in the RESET CeD Study IL-2 response to screening gluten challenge was not an eligibility criterion, this new tool could be used in future studies to avoid enrolling patients who may be misdiagnosed with CeD who do not show elevated IL-2.

In common with several recent studies, a strength of the RESET CeD Study was that it utilized quantitative histomorphometry as opposed to qualitative classifications, which have lower reliability and reproducibility, and this allowed significant improvement in duodenal histology to be detected in some Nexvax2-treated patients but none of the placebo group. A recently reported large mRNA panel to assess gene expression in duodenal biopsies may also have added further understanding to the effects of Nexvax2 on tissue response in gut.[30] Similarly, assessing a wider range of serum cytokines such as IL-10 and interferon-γ-dependent chemokines CXCL-9 and CXCL-10 during the hours after bolus gluten challenge may have provided more insight than assessing IL-2 alone.[14]

The RESET CeD Study highlights the difficulties of developing and testing SIT in a disease that is driven by specific proinflammatory CD4+ T cells when the native antigen is insoluble and the peptides recognized by these CD4+ T cells are diverse. The RESET CeD Study suggests that restoration of immune tolerance is graded and modification of symptoms caused by gluten may be more difficult to demonstrate than histology and immune endpoints. For the class of immunotherapy utilizing peptides alone, the RESET CeD Study suggests suitable peptides regularly administered may positively impact tissue injury in organ-specific immune disease, but may be unable to modify the effects of acute, heavy antigen exposure. This study highlights the importance of carefully calibrated gluten challenge to assess efficacy of novel therapies in CeD, and emphasises the importance of designing clinical trials to address the intended indication

whether to control low gluten exposure on GFD or to powerfully suppress gluten immunity allowing an unrestricted diet.

Declaration of interest statement: GG, KEG, HLH, KMN, KET, LJW, and RPA were formerly employees of ImmusanT, Inc. JAT-D, MM and AJMD served as advisors to ImmusanT, Inc. JT-D reports grants from ImmusanT Inc., during the conduct of the study; grants from Chugai Pharmaceuticals, grants from Novoviah Pharmaceuticals, grants from Tillots Pharmaceuticals, grants from Codexis, personal fees from Janssen , personal fees from Anokion, personal fees from Codexis, personal fees from Chugai, personal fees from Mozart Therapeutics, outside the submitted work; in addition, Dr. Tye-Din has a patent PCT/AU2009/001556 licensed to ImmusanT Inc, and a patent PCT/GB2005/001621 licensed to ImmusanT Inc. AJMD reports personal fees from ImmusanT, Inc. during the conduct of the study. AP, JT, and JI report personal fees from Jilab during the conduct of the study. MM reports personal fees outside the current work from Dr Falk Pharma, Calypso Biotech and Topas Therapeutics, non-financial support from Immunogenix; MM also receives royalties from patents US7,361,480 and EU1390753. JI, AL and JT report personal fees from Jilab during the conduct of the study. RPA has patents relating to therapy and diagnosis of coeliac disease licensed to ImmusanT. The other authors declared no conflicts of interest.

Contributions JAT-D: Resources, Investigation, Writing – review & editing; AJMD: Resources, Investigation, Writing – review & editing; GG: Conceptualization, Data curation, Formal Analysis, Methodology, Software, , Writing – review & editing; KEG: Methodology, Project administration, Writing – review & editing; HLH: Project administration, Writing – review & editing; KMN: Project administration, Visualization, Writing – original draft, Writing – review & editing; AP: Investigation, Writing – review & editing; JT: Investigation, Writing – review & editing; MM: Resources, Investigation, Writing – review & editing; JI: Resources, Investigation, Writing – review & editing; LJW: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing; KET: Conceptualization, Project administration, Supervision, Writing – review & editing; RPA: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Methodology, Supervision, Visualization, Writing – original draft, and revisions, Writing – review & editing; RESET CeD Study Group: Resources, Investigation.

Data sharing statement Data collected for the study is not available to others.

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Figure legends

Figure 1. Patient Disposition

Figure 2. Change in IL-2 serum concentration from within 30 minutes before to 4 hours after the first masked gluten food challenge (MFC2) at Visit 34 for Nexvax2-treated subjects (a) to (c) (n = 31) and placebo-treated subjects (d) to (f) (n = 32) with available data. Panel (a) and (d) compare serum IL-2 at 4 h after unmasked gluten screening food challenge (SFC) with masked sham food challenge at Visit 28 to masked gluten food challenge at Visit 34 (median indicated) with statistical significance tested by ANCOVA ** $P < 0.01$, *** $P < 0.001$, ns not significant; (b) and (e) compare unmasked gluten screening food challenge (SFC) to masked gluten food challenge at Visit 34 for individual subjects, correlation tested by Spearman test; and (c) and (f) compare change from baseline in Total Gastrointestinal score for the day of masked gluten food challenge at Visit 34 to change in IL-2 serum concentration from within 30 minutes before to 4 hours for individual subjects, correlation tested by Spearman test.

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Table 1: Demographics of all non-homozygotes randomised and those with CeD PRO data available for primary efficacy endpoint analysis in the intention-to-treat population

	Non-homozygous HLA-DQ2.5		All available for primary endpoint analysis	
	Nexvax2 group (n=76)	Placebo group (n=78)	Nexvax2 group (n=33)	Placebo group (n=34)
Mean (SD) age in years	42 (14)	43 (15)	42 (14)	46 (13)
Number (%) females	59 (77%)	60 (77%)	27 (82%)	26 (76%)
Mean (SD) height in centimeters	169 (10)	169 (10)	168 (10)	168 (10)
Mean (SD) body mass in kilograms	77 (17)	81 (20)	77 (15)	80 (21)
Mean (SD) body mass index	27 (6)	28 (7)	27 (5)	28 (7)
Number (%) White, not Hispanic or Latino	76 (100%)	77 (99%)	30 (91%)	34 (100%)
Median (interquartile range) age at diagnosis	33 (25 - 44)	36 (25 - 47)	31 (24-42)	41 (33-48)
Median (interquartile range) years duration CeD	7 (3 -11)	6 (3 - 10)	7 (4-11)	6 (3-10)
Number (%) negative for both CeD serologies†	60 (79%)	74 (95%)	27 (82%)	31 (91%)
Number (%) positive for both CeD serologies†	3 (4%)	0 (0%)	2 (6%)	0 (0%)
Number (%) IgA deficient (<7 mg/dL)	1 (1%)	0 (0%)	1 (3%)	0 (0%)
Number (%) recruited in United States	34 (45%)	32 (41%)	16 (48%)	20 (59%)
Number (%) recruited in Australia	28 (37%)	37 (47%)	7 (21%)	11 (32%)
Number (%) recruited in New Zealand	14 (18%)	9 (12%)	10 (30%)	3 (9%)

† QUANTA Lite® R h-tTG IgA (normal range: 3 U/mL or less) and Gliadin IgA II, INOVA Diagnostics (normal range: 19 U or less)

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Table 2: Primary and secondary symptom-based endpoints: Mean (standard deviation) change baseline to Visit 34 daily CeD PRO compared by ANCOVA

	Nexvax2 Group	Placebo Group	MD (95% CI); P†
Patients (n)	33	34	
	Primary endpoint		
Total gastrointestinal score	2.86 (2.28)	2.63 (2.07)	0.41 (-0.62, 1.44); 0.43
	Secondary endpoints		
Abdominal domain	2.73 (2.17)	1.86 (1.58)	1.03 (0.15, 1.93); 0.02
Diarrhea loose stool domain	3.36 (3.01)	3.35 (3.21)	0.17 (-1.38, 1.72); 0.83
Cramping	2.60 (2.69)	1.79 (2.07)	1.09 (-0.06, 2.24); 0.06
Pain	2.79 (2.87)	1.85 (2.13)	0.97 (-0.28, 2.22); 0.13
Bloating	3.06 (3.01)	1.79 (3.36)	1.48 (0.32, 2.64); 0.01
Diarrhea	1.88 (2.91)	2.14 (3.14)	0.00 (-1.50, 1.49); 0.99
Gas	2.45 (2.29)	2.00 (1.92)	0.70 (-0.22, 1.61); 0.14
Loose stool	4.85 (3.94)	4.56 (4.01)	0.33 (-1.65, 2.30); 0.74
Nauesa	2.48 (3.10)	2.68 (3.10)	-0.20 (-1.67, 1.27); 0.79

†ANCOVA analyses used to calculate Mean difference (95%CI); P-value.

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Table 3: Summary of TEAEs during the treatment period that occurred in at least 10% and two or more patients in any group (intention-to-treat population)

	Non-homozygous HLA-DQ2.5		Homozygous HLA-DQ2.5	
	Nexvax2 group (n=76)	Placebo group (n=78)	Nexvax2 group (n=16)	Placebo group (n=8)
Patients with at least one TEAE	68 (89%)	72 (92%)	16 (100%)	8 (100%)
Patients with at least one treatment-related* TEAE	60 (79%)	59 (76%)	14 (88%)	8 (100%)
Patients who discontinued because of adverse events	1 (1%)	1 (1%)	0	0
Patients with any serious adverse event	1 (1%)	6 (8%)	0	0
Patients with any TEAE related to gluten challenge	36 (47%)	41 (53%)	14 (88%)	5 (63%)
Patients with any TEAE in updosing (Visit 5 - 16)	67 (88%)	68 (87%)	15 (94%)	7 (88%)
Number of TEAE	793	820	269	88
Number of TEAE graded severe	47	51	14	1
Number of TEAE graded moderate	220	238	94	42
Number of TEAE graded mild	526	531	161	45
Number of treatment-related TEAE's	463	477	152	46
Number of TEAEs related to gluten challenge	125	181	59	23
Number of TEAE in updosing (Visit 5 - 16)	414	414	117	45
Patients with treatment-related TEAE†:				
Patients with at least one treatment-related* TEAE	60 (79%)	59 (76%)	14 (88%)	8 (100%)
Patients with a “Gastrointestinal disorders” TEAE	50 (66%)	52 (67%)	15 (94%)	8 (100%)
Patients with nausea	35 (46%)	25 (32%)	9 (56%)	4 (50%)
Patients with diarrhoea	25 (33%)	24 (30%)	7 (44%)	1 (13%)
Patients with abdominal pain	23 (30%)	21 (27%)	8 (50%)	6 (75%)
Patients with abdominal distension	11 (14%)	15 (19%)	6 (38%)	3 (38%)
Patients with vomiting	7 (9%)	6 (8%)	1 (6%)	2 (25%)
Patients with flatulence	6 (8%)	4 (5%)	3 (19%)	2 (25%)
Patients with gastro-oesophageal reflux disease	2 (3%)	1 (1%)	2 (13%)	0 (0%)
Patients with abdominal discomfort	3 (4%)	2 (3%)	2 (13%)	1 (0%)
Patients with a “Nervous system disorders” TEAE	30 (39%)	26 (33%)	6 (38%)	3 (38%)
Patients with headache	25 (33%)	17 (22%)	7 (44%)	3 (38%)
Patients with migraine	2 (3%)	3 (4%)	2 (13%)	0 (0%)
Patients with a “General disorders and administration site conditions” TEAE	30 (39%)	30 (38%)	5 (31%)	6 (75%)
Patients with fatigue	20 (26%)	26 (33%)	4 (25%)	5 (63%)
Patients with injection site bruising	4 (5%)	4 (5%)	1 (6%)	1 (13%)

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 probably, and possibly related to the study drug (none were considered definitely related

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Table 4: Mean (standard deviation) change from baseline to end-of-study in quantitative second-part duodenal histology compared by ANCOVA

	Nexvax2		Placebo		MD (95%CI); P†
Patients, n	13		14		
	Baseline	EOS	Baseline	EOS	
VH:CrD	1.67 (0.45)	1.80 (0.35)	1.66 (0.35)	1.57 (0.27)	0.22 (0.06, 0.39); 0.01
VH	319.8 (53.0)	339 (33.8)	321.1 (44.0)	305.4 (39.4)	34.4 (14.6, 54.1); 0.001
CrD	199.3 (38.1)	192.9 (29.3)	197.4 (26.5)	197.1 (22.2)	-5.2 (-21.0, 10.7); 0.51
VH + CrD	519.2 (53.3)	531.9 (46.0)	518.5 (47.6)	502.4 (46.2)	29.1 (1.7, 56.5); 0.04
IELs	34.1 (4.7)	35.9 (10.1)	28.5 (6.4)	35.9 (10.0)	-2.1 (-11.1, 6.9); 0.64

†ANCOVA models used to calculate Mean difference (95%CI); P-value. End-of-study (EOS), Villus height, µm (VH), crypt depth, µm (CrD), IELs, CD3-positive intraepithelial lymphocytes per 100 epithelial cells

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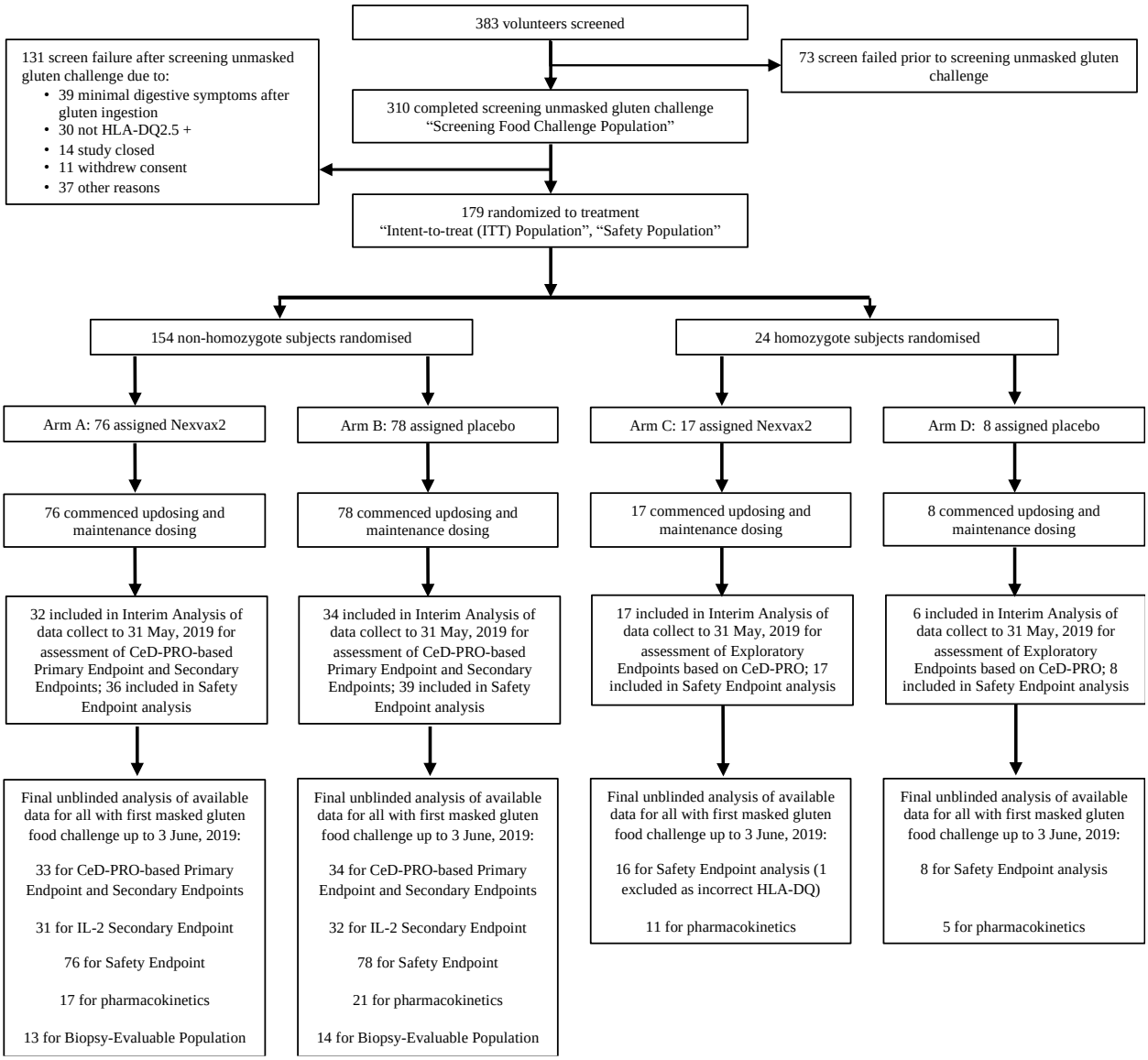

Figure 1. Patient Disposition

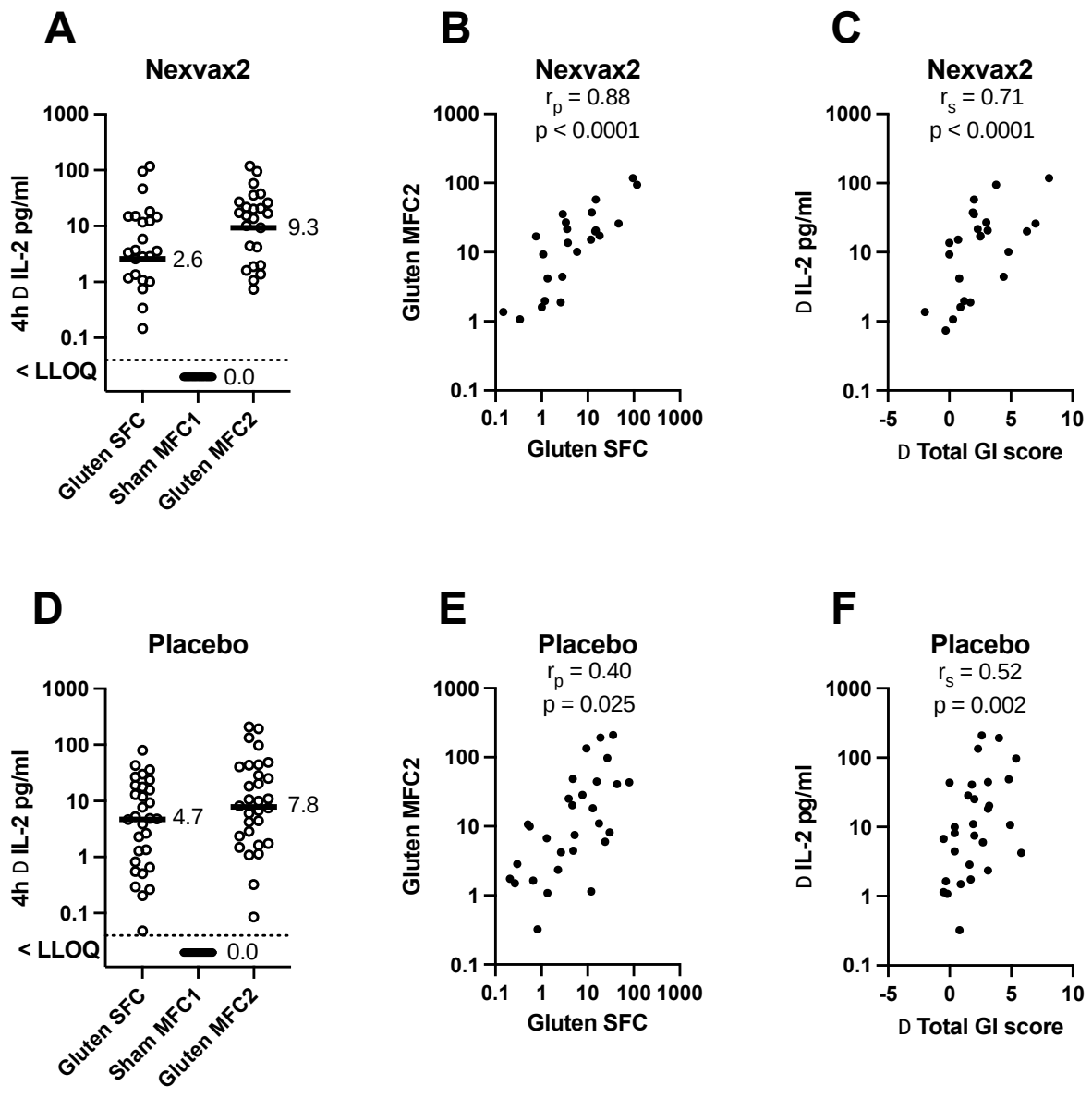

Figure 2. Change in IL-2 serum concentration from within 30 minutes before to 4 hours after the first masked gluten food challenge (MFC2) at Visit 34 for Nexvax2-treated subjects (a) to (c) (n = 31) and placebo-treated subjects (d) to (f) (n = 32) with available data. Panel (a) and (d) compare serum IL-2 at 4 h after unmasked gluten screening food challenge (SFC) with masked sham food challenge at Visit 28 to masked gluten food challenge at Visit 34 (median indicated) with statistical significance tested by ANCOVA ** P < 0.01, *** P < 0.001, ns not significant; (b) and (e) compare unmasked gluten screening food challenge (SFC) to masked gluten food challenge at Visit 34 for individual subjects, correlation tested by Spearman test; and (c) and (f) compare change from baseline in Total Gastrointestinal score for the day of masked gluten food challenge at Visit 34 to change in IL-2 serum concentration from within 30 minutes before to 4 hours for individual subjects, correlation tested by Spearman test.

Appendix

Efficacy and safety of gluten peptide-based antigen-specific immunotherapy (Nexvax2) in adults with coeliac disease after bolus exposure to gluten: a phase 2 randomised, double-blind, placebo-controlled study

A. Prof. Tye-Din, Jason A.; A. Prof. Daveson, A James M.; Goel, Gautam; Goldstein, Kaela E.; Hand, Holly L.; Neff, Kristin M.; Popp, Alina; Taavela, Juha; Prof. Maki, Markku, Prof. Isola, Jorma, Williams, Leslie J.; Truitt, Kenneth E.; Anderson, Robert P.* on behalf of the RESET CeD Study Group

Supplementary Materials and Methods

Investigational Drug Product

Nexvax2 Sterile Solution for Injection (1.5 mg/mL in 0.9% sodium chloride) is an equimolar solution of 3 peptides (NPL001, NPL002, and NPL003) and is described elsewhere.[1] Grand River Aseptic Manufacturing (Grand Rapids, Michigan, USA) manufactured Nexvax2 or placebo (sodium chloride United States Pharmacopeia [USP] 0.9%) in vials with a 1.3 mL fill volume and in BD Neopak™ syringes with a 0.6 mL fill volume. The pre-filled vials were used with diluent (sodium chloride United States Pharmacopeia [USP] 0.9%) to prepare 11 fixed doses ranging from 1 μg to 750 μg for dose escalation. The pre-filled Neopak syringes (900 μg) were encased in a PhysioJect disposable autoinjector for use during the maintenance phase of the study. Catalent Pharma Solutions (Somerset, NJ, United States) were responsible for shipment of double-blind treatment kits to the study site. The appearance of study drug vials and autoinjectors, the study drug itself, the volume injected, and the number of injections for Nexvax2 and placebo treatments were identical within each cohort.

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Table S1. Study sites, principal investigators, enrollment, and randomization†

1. The Royal Melbourne Hospital - The Walter And Eliza Hall Institute of Medical Research (Parkville, VIC, Australia) (Site 308) – Dr. Jason Tye-Din. 34 enrolled for screening food challenge, 24 randomised
2. The Wesley Hospital - The Wesley Research Institute (Auchenflower, QLD, Australia) (Site 309) – Dr. A. James Daveson. 35 enrolled for screening food challenge, 20 randomised
3. P3 Research Limited (Wellington, New Zealand) (Site 203) – Dr. Dean Quinn. 18 enrolled for screening food challenge, 11 randomised
4. Advanced Research Institute (South Ogden, UT, USA) (Site 115) – Dr. John Lowe. 17 enrolled for screening food challenge, 11 randomised
5. Auckland Clinical Studies Ltd. (Auckland, New Zealand) (Site 202) – Dr. Timothy King. 18 enrolled for screening food challenge, 9 randomised
6. AB Clinical Trialss (Las Vegas, NV, USA) (Site 103) – Dr. Atoya Adams. 11 enrolled screening food challenge, 8 randomised
7. Clinical Trials Centre - University of the Sunshine Coast (Sippy Downs, QLD, Australia) (Site 303) – Dr. Susan Thackwray. 17 enrolled for screening food challenge, 8 randomised
8. Coral Sea Clinical Research Institute (Mackay, QLD, Australia) (Site 306) – Dr. A. James Daveson. 12 enrolled for screening food challenge, 7 randomised
9. PMG Research of McFarland Clinic (Ames, IA, USA) (Site 128) – Dr. Bryan Feyen. 11 enrolled for screening food challenge, 6 randomised
10. Gastroenterology Associates of Western Michigan, PLC d.b.a. West Michigan Clinical Research Center (Wyoming, MI, USA) (Site 127) – Dr. Allan Coates. 8 enrolled for screening food challenge, 6 randomised
11. Royal Adelaide Hospital (Adelaide SA, Australia) (Site 305) – Dr. Jane Andrews. 11 enrolled for screening food challenge, 5 randomised
12. Long Island Gastrointestinal Research Group (Great Neck, NY, USA) (Site 107) – Dr. Michael Goldstein. 10 enrolled for screening food challenge, 5 randomised
13. University of Iowa (Iowa City IA, USA) (Site 122) – Dr. David Elliott. 7 enrolled for screening food challenge, 5 randomised
14. P3 Research Limited (Havelock North, New Zealand) (Site 204) – Dr. Richard Stubbs. 6 enrolled for screening food challenge, 5 randomised
15. Omega Medical Research (Warwick, RI, USA) (Site 118) – Dr. Eric Newton. 9 enrolled for screening food challenge, 4 randomised
16. Diablo Clinical Research, Inc. (Walnut Creek, CA, USA) (Site 108) – Dr. Helen Stacey. 7 enrolled for screening food challenge, 4 randomised
17. Texas Digestive Disease Consultants (Southlake, TX, USA) (Site 116) – Dr. Timothy Ritter. 6 enrolled for screening food challenge, 4 randomised
18. Sir Charles Gairdner Hospital (Nedlands, WA, Australia) (Site 307) – Dr. Hooi Ee. 7 enrolled for screening food challenge, 3 randomised
19. Heartland Research Associates, LLC (Wichita, KS, USA) (Site 105) – Dr. Thomas Klein. 6 enrolled for screening food challenge, 3 randomised
20. Eastern Health-Box Hill Hospital (Box Hill, VIC, Australia) (Site 301) – Dr. Sweelin Chen Yi Mei. 4 enrolled for screening food challenge, 3 randomised
21. Drug Trials America (Hartsdale, NY, USA) (Site 111) – Dr. Michael Gerdis. 4 enrolled for screening food challenge, 3 randomised
22. Mayo Clinic (Rochester, MN, USA) (Site 120) – Dr. Joseph Murray. 3 enrolled for screening food challenge, 3 randomised
23. Thomas Jefferson University Hospital (Philadelphia, PA, USA) (Site 114) – Dr. Anthony DiMarino. 3 enrolled for screening food challenge, 3 randomised
24. Gastroenterology and Endoscopy Specialists (Christchurch, New Zealand) (Site 201) – Dr. Richard Gearry. 4 enrolled for screening food challenge, 2 randomised
25. Center for Digestive Health (Troy, MI, USA) (Site 117) – Dr. John Weber. 4 enrolled for screening food challenge, 2 randomised
26. ActivMed Practices & Research, Inc. (Portsmouth, NH, USA) (Site 106) – Dr. Roger Epstein. 4 enrolled for screening food challenge, 2 randomised
27. Digestive Health Research, LLC (Hermitage, TN, USA) (Site 121) – Dr. George James.4 enrolled for screening food challenge, 2 randomised
28. Alfred Hospital (Melbourne, VIC, Australia) (Site 302) – Dr. Gregor Brown. 3 enrolled for screening food challenge, 2 randomised
29. Great Lakes Gastroenterology Research, LLC (Mentor, OH, USA) (Site 104) – Dr. Keith Friedenberg. 3 enrolled for screening food challenge, 2 randomised
30. Coastal Carolina Research Center (Mount Pleasant, SC, USA) (Site 110) – Dr. Cynthia Strout. 2 enrolled for screening food challenge, 2 randomised
31. Allegiance Research Specialists (Wauwatosa, WI, USA) (Site 101) – Dr. Samuel Idarraga. 3 enrolled for screening food challenge, 1 randomised
32. Stamford Therapeutics Consortium (Stamford, CT, USA) (Site 132) – Dr. David Radin. 3 enrolled for screening food challenge, 1 randomised
33. Alliance Medical Research LLC (Lighthouse Point, FL, USA) (Site 124) – Dr. Vipin Gupta. 3 enrolled for screening food challenge, 1 randomised

34. PMG Research of Winston-Salem, LLC (Winston-Salem, NC, USA) (Site 119) – Dr. Robert Holmes.3 enrolled for screening food challenge, 1 randomised
35. Ocean State Clinical Research Partners (Lincoln, RI, USA) (Site 125) – Dr. Scott Wilson. 2 enrolled for screening food challenge, 1 randomised
36. Clinical Research Insitute of Michigan (Chesterfield, MI, USA) (Site 102) – Dr. Ronald Fogel. 4 enrolled screening food challenge, 0 randomised to treatment
37. The University of Queensland - Princess Alexandra Hospital (Woolloongabba, QLD, Australia) (Site 304) – Dr. Gerald Holtmann. 2 enrolled for screening food challenge, 0 randomised
38. PMG Research, Inc., d/b/a PMG Research of Piedmont Healthcare (Statesville, NC, USA) (Site 129) – Dr. Vivek Trivedi. 1 enrolled for screening food challenge, 0 randomised
39. Grand Teton Research Group, PLLC (Idaho Falls, ID, USA) (Site 109) – Dr. Clint Behrend. 1 enrolled for screening food challenge, 0 randomised

† Sites that did not enroll for screening food challenge or randomize any patients are not shown
United States of America (139 enrolled for screening food challenge, 80 randomised), Australia (125 enrolled for screening food challenge, 72 randomised) and New Zealand (46 enrolled for screening food challenge, 27 randomised)

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Table S2. Study Independent Ethics Committees and approvals

United States of America

1. Copernicus Group IRB, 5000 Centre Green Way, Suite 200, Cary NC, 27513, Study Number 20181291
2. Columbia University IRB, 154 Haven Avenue, 1st Floor, New York NY, 10032, Study Number IRB-AAAR9068
3. Mayo Clinic IRB, 200 First Street SW, Rochester MN, 55905, Study Number 18-004575
4. The University of Chicago IRB, 5841 South Maryland Avenue, MC7132, I-625, Chicago IL, 60637, Study Number IRB18-1200
5. Western IRB, 1019 39th Ave SE, Puyallup WA, 98374, Study Number 20181291

New Zealand

6. Central Health and Disability Ethics Committee, Ministry of Health, 133 Molesworth St, Thorndon, Wellington, 6011, Study Number 18/SCOTT/70

Australia

7. Bellberry Limited, 129 Glen Osmond Road, Eastwood SA, 5063, Application Number 2018-07-562-A-13
8. Melbourne Health Human Research Ethics Committee, Office for Research, Level 2 South West, 300 Gratan Street, Parkville VIC, 3050, Study Number HREC/43048/MH-2018
9. Uniting Care Health, 129 Glen Osmond Road, Eastwood SA, 5063, Study Number 1818

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Table S3. Eligibility criteria

To be eligible to participate, volunteers must have met the following inclusion criteria and none of the exclusion criteria at the first study visit or at the time indicated.

At Screening

Inclusion Criteria

1. Adults 18 to 70 years of age (inclusive) who have signed an informed consent form.
2. History of medically diagnosed coeliac disease that included assessment of duodenal biopsies.
3. Initiated gluten free diet at least 12 months prior to screening.
4. No known allergy or hypersensitivity to any ingredients, except gluten, in the products used for the food challenges (potato protein, rice starch, guar gum, and fruit drink flavoring [beet juice, elderberry juice, crystallized lime, and stevia]).
5. Willingness to consume food containing up to 6 grams of gluten protein at one time and up to 18 grams of gluten protein in total during the study (including screening).
6. Willingness to undergo study procedures.
7. Able to read and understand English.

Exclusion Criteria

1. Refractory coeliac disease according to "The Oslo definitions for coeliac disease and related terms" (i.e., persistent or recurrent malabsorptive symptoms and signs with villous atrophy despite a strict gluten free diet for more than 12 months).
2. History of inflammatory bowel disease and/or microscopic colitis.
3. Any medical condition or other reason that in the opinion of the investigator may interfere with study conduct.
4. Any medical condition that in the opinion of the investigator would impact the immune response (other than coeliac disease), confound interpretation of study results, or pose an increased risk to the patient.
5. Unable or unwilling to perform self-administration of investigational product.
6. Use of immunomodulatory or immune-suppressing medical treatment during the 6 months prior to the first day of screening (e.g., azathioprine, methotrexate, or biological).
7. Use of oral or parenteral immunomodulatory corticosteroids, including budesonide, within the 6 to 9 weeks prior to the first day of screening. Topical or inhaled corticosteroids are acceptable.
8. Dosing with placebo or active IP in a clinical study with Nexvax2.
9. Receipt of any investigational drug in another clinical study within 6 months prior to the first day of screening.
10. Females who are lactating or pregnant, including those with positive urinary pregnancy test on the first day of screening.

At Randomization

Inclusion Criteria

1. A history of coeliac disease diagnosed on the basis of duodenal biopsy showing villous atrophy and abnormal coeliac disease-specific serology (e.g., anti-TG2 IgA), which predate screening and confirm diagnosis.
2. Positive for the HLA-DQ2·5 genotype. (Note: only patients with two copies of both the HLA-DQA1*05 and HLA-DQB1*02 alleles are considered homozygotes. Randomization into the corresponding HLA-DQ2·5 non-homozygous and homozygous cohort will be tracked centrally and capped.)
3. An increase of at least 3 in the global symptom survey numerical score at any timepoint from 2 hours to 6 hours post-SFC when compared to pre-screening food challenge global symptom survey, or a gastrointestinal adverse event of at least moderate severity following the screening food challenge, up to midnight on the day of the screening food challenge.

Exclusion Criteria

1. Receipt of any vaccine (e.g., influenza) within 1 week prior to the planned first day of the treatment period.
2. Presence of 1 or more of the following laboratory abnormalities at screening:
    - alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, or gamma-glutamyltransferase > 2.0 × the upper limit of normal.
    - total bilirubin > 2.0 × upper limit of normal or direct bilirubin > 1.0 × upper limit of normal.
    - serum creatinine > 1.5 × upper limit of normal.
    - hemoglobin levels < 10 g/dL.
    - platelet count < 75×10^9/L.
    - neutrophil count < 1.5×10^9/L (i.e., < 1500/mm^3).
    - Thyroid-stimulating hormone outside the normal range and judged clinically significant by the investigator.
    - White blood cell count outside the normal range and judged clinically significant by the investigator
3. The patient misses 5 or more of the daily CeD PRO assessments during the 14-day baseline during the screening period immediately before the first dose of investigational product.

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Table S4. Schedule of assessments

	Screening Period			Treatment Period: Uptitling Phase												Treatment Period: Maintenance Phase (First Part)																		Treatment Period: Maintenance Phase (Continued)									Observational Follow-up Phase	
Visit	V1 SFC[1]	V2	V-EGD1	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16	V17	V18	V19	V20	V21	V22	V23	V24	V25	V26	V27	V28 (MFC1)	V29	V30	V31	V32	V33	V34 (MFC2)	V35	V36	V37	V38	V39	V40 (MFC3)	V41	V42 (EOT/ET)	V43 (EOS)
Day	-83 to -42	-21	-21 to -14	-14	-7	1	4	8	11	15	18	22	25	29	32	36	39	43	46	50	53	57	60	64	67	71	74	78	79	80	81	85	88	92	93	94	95	99	102	106	107	108	113	120±2	141±3
Week	-9 to -6	-3	-3 to -2	-2	-1	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8	8	9	9	10	10	11	11	12	12	12	12	13	13	14	14	14	14	15	15	16	16	16	17	18	21[b]
Nexus2 Arm A & C) (qd						1	3	9	30	60	90	150	300	450	600	750	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900	900		
Placebo (Arm B & D) (qd						0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
Dose Number						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23			24	25	26	27			28	29	30	31		32		
Visit Locations																																												
Study Site	X		(X)		X		X	X	X	X	X	X	X	X	X	X	X			Phone						X					X					X		X		X	(X)	X
Patient's Home		X		X		X												X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Administrative Procedures																																											
Informed Consent	X																																										
Inclusion/Exclusion Criteria	X																																										
Randomization						X																																					
Demographics		X				X																																					
Medical/Surgical History		X																																									
Celiac Disease Diagnosis		X																																									
Clinical Characteristics of Celiac Disease		X																																									
Prior Concomitant Medications		X		(X)			X	X	X	X	X	X	X	X	X	X	X				X													X						X		X
CDAT	X[c]																																										
Compliance with GFD	X[c]		(X)				X	X	X	X	X	X	X	X	X	X	X	X	X		X						X		X					X						X	(X)	X
EFD	X[c]																																										
HLA-DQ8, and HLA-DQB	X																																										
GLOSS[d]	X, 1h, 2h, 3h, 4h, 5h, 6h																																										
Modified CeD PRO[3,6]	X, 1h, 2h, 3h, 4h, 5h, 6h																																										
Clinical Procedures																																											
Vital Signs	X[e]					X, 4h	X	X	X	X	X	X	X	X	X	X	X, 4h					X						X, 4h					X, 4h					X, 4h				
Weight	X																																										
Height	X																																										
Physical Examination	X																																									X	
12-lead ECG	X					X																				X															X	
Adverse Event Monitoring	X[e]		(X)			X	X	X	X	X	X	X	X	X	X	X	X			X						X		X					X					X		X	(X)	X
Endoscopy/Duodenal Biopsys			(X)																																							(X)	
Clinical Outcome Assessments																																											
Provide and/or Collect ePRO Device	X[f]					X[e]																																					X
Daily CeD PRO[3]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
IBFS + PGA-IBF[5]		X	(X)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECA-S[3]					X												X	X[e]								X[e]				X[e]					X[e]					X		X
CGA-S[3]						X[e]											X	X[e]								X[e]				X[e]					X[e]					X		X
EPDS[3]					X													X								X															X		X
SF-12v2[3]					X												X									X																	X
Laboratory Assessments																																											
Hematology/Coagulation	X					X											X												X													X	
Blood Chemistry 1	X					X											X												X													X	
Blood Chemistry 2	X					X											X												X													X	
Urinalysis	X					X											X												X													X	
Urine Pregnancy Test	X					X																							X													X	
Serum Pregnancy Test	X																																										
Serum Celiac Disease Serology	X					X											X												X													X	
Exposure Pharmacokinetics[g]																	X, 45m												X													X, 45m	
Serum Anti-Nexus2 Antibodies						X											X																									X	
Serum Cytokines[i]	X[e], 2h, 4h, 6h					X, 4h											X, 4h																	X, 4h					X, 4h		X, 4h		
Blood Sampling for Exploratory Immune-Cell Studies (selected sites only)						X																							X														X
Administration of IP																																											
IP Administration by Site Staff						X	X	X	X	X	X	X	X	X	X	X																											
Patient Self-administration of IP																	X																X	X			X	X	X		X		
Dispense Auto-injectors																	X																										
Return Used Auto-injectors																																		X[e]						X[e]			
PC Procedure																																											
Unmasked Gluten PC (SFC)	X[c]																																											
Masked Gluten/Sham PC (MFC)																													X											X			

Table S5: Mean (standard deviation) baseline CeD PRO scores for all HLA-DQ2.5 non-homozygous patients randomized (intention to treat, ITT) and those included in primary endpoint analysis who completed V34 with PRO data

	Nexvax2		Placebo	
	ITT	Completed V34 with PRO data	ITT	Completed V34 with PRO data
Patients (n)	77	33	78	34
Total Gastrointestinal Score†	0.5 (0.5)	0.5 (0.6)	0.8 (0.9)	0.9 (1.3)
Abdominal Domain	0.6 (0.6)	0.8 (0.8)	1.0 (1.1)	1.1 (1.3)
Diarrhea and Loose Stool Domain	0.3 (0.5)	0.5 (0.8)	0.6 (1.3)	0.9 (1.4)
Total Non-gastrointestinal Domain	1.0 (1.0)	1.2 (1.0)	1.3 (1.3)	1.3 (1.4)
Cramping	0.3 (0.4)	0.4 (0.5)	0.7 (1.1)	0.8 (1.4)
Pain	0.3 (0.5)	0.5 (0.6)	0.7 (1.1)	0.8 (1.2)
Bloating	0.7 (1.0)	1.1 (1.2)	1.1 (1.4)	1.3 (1.7)
Diarrhea	0.3 (0.7)	0.4 (0.8)	0.7 (1.3)	0.9 (1.6)
Gas	1.0 (1.1)	1.2 (1.3)	1.4 (1.2)	1.5 (1.4)
Loose Stool	0.6 (1.0)	0.6 (0.9)	0.8 (1.2)	0.9 (1.5)
Nausea	0.3 (0.4)	0.3 (0.5)	0.6 (1.4)	0.7 (1.6)
Headache	0.6 (0.9)	0.6 (0.9)	0.7 (1.0)	0.6 (1.1)
Tiredness	1.5 (1.5)	1.8 (1.6)	1.8 (1.7)	1.9 (1.8)

Table S6: Mean (standard deviation) of CeD PRO scores for key visit days in HLA-DQ2.5 non-homozygous patients who completed Visit 34

	Nexvax2 group					Placebo group				
Visit	BSL	V5	V16	V28	V34	BSL	V5	V16	V28	V34
Data available (n)	33	30	30	33	33	34	31	32	32	34
NX2 (μg)	-	1	900	-	-	-	-	-	-	-
Gluten	-	-	-	-	MFC2	-	-	-	-	MFC2
Sham	-	-	-	MFC1	-	-	-	-	MFC1	-
Domain scores (0 – 10)										
Total	0.5	0.8	0.6	0.6	3.2	0.9	0.9	0.9	0.8	2.9
Gastrointestinal†	(0.6)	(1.2)	(0.8)	(0.7)	(2.5)	(1.3)	(1.0)	(1.0)	(1.4)	(2.4)
Abdominal	0.8	1	0.9	1.1	2.7	1.1	1.2	1.1	0.8	1.9
	(0.8)	(1.5)	(1.0)	(1.2)	(2.2)	(1.3)	(1.4)	(1.2)	(1.1)	(1.6)
Diarrhoea & Loose	0.5	0.6	0.6	0.3	2.2	0.9	0.7	0.4	0.9	2.4
stool	(0.8)	(1.7)	(1.6)	(1.0)	(2.9)	(1.4)	(1.4)	(1.1)	(2.0)	(3.3)
Total non-	1.2	1.7	1.8	2.1	3.3	1.3	1.4	1.9	1.3	3.3
Gastrointestinal	(1.0)	(1.9)	(1.5)	(2.2)	(2.7)	(1.4)	(1.2)	(1.6)	(1.6)	(2.6)
Individual symptom severity scores (0 – 10)										
Cramping	0.4	0.8	0.5	0.6	2.6	0.8	0.9	0.8	0.5	1.8
	(0.5)	(1.9)	(1.3)	(1.4)	(2.7)	(1.4)	(1.6)	(1.4)	(1.2)	(2.1)
Pain	0.5	0.7	0.6	0.5	2.8	0.8	0.8	0.8	0.5	1.9
	(0.6)	(1.8)	(1.2)	(1.2)	(2.9)	(1.2)	(1.6)	(1.5)	(1.0)	(2.1)
Bloating	1.1	1.1	1.1	1.3	3.1	1.3	1.3	1.3	0.8	1.8
	(1.2)	(2.0)	(1.5)	(1.8)	(3.0)	(1.7)	(2.0)	(1.4)	(1.4)	(2.4)
Diarrhea	0.4	0.5	0.4	0.2	1.9	0.9	0.7	0.4	1.0	2.1
	(0.8)	(1.7)	(1.6)	(0.6)	(2.9)	(1.6)	(1.6)	(1.2)	(2.2)	(3.1)
Gas	1.2	1.3	1.6	1.8	2.5	1.5	1.8	1.6	1.5	2.0
	(1.3)	(1.5)	(1.6)	(1.7)	(2.3)	(1.4)	(1.8)	(1.7)	(1.6)	(3.9)
Loose Stool	0.6	0.7	0.7	0.5	2.4	0.9	0.7	0.3	0.8	2.6
	(0.9)	(1.7)	(1.7)	(1.3)	(3.3)	(1.5)	(1.4)	(1.2)	(2.0)	(3.5)
Nausea	0.3	0.8	0.3	0.3	4.8	0.7	0.7	1.2	0.6	4.6
	(0.5)	(1.6)	(0.7)	(0.7)	(3.9)	(1.6)	(1.5)	(2.1)	(1.6)	(4.0)
Headache	0.6	0.8	1	1.3	2.5	0.6	0.8	0.8	0.7	2.7
	(0.9)	(1.8)	(1.5)	(2.3)	(3.1)	(1.1)	(1.2)	(1.2)	(1.2)	(3.1)
Tiredness	1.8	2.6	2.6	2.9	4.2	1.9	1.9	2.9	2.0	3.8
	(1.6)	(2.7)	(2.1)	(2.7)	(3.0)	(1.8)	(1.9)	(2.7)	(1.5)	(2.9)

Mean (standard deviation) for the visit day, or the average over the 14 days before Visit 5 for baseline (BSL); MFC masked food challenge

†Total Gastrointestinal Score is one third the sum of the scores for Nausea, Abdominal Domain and Diarrhoea & Loose stool

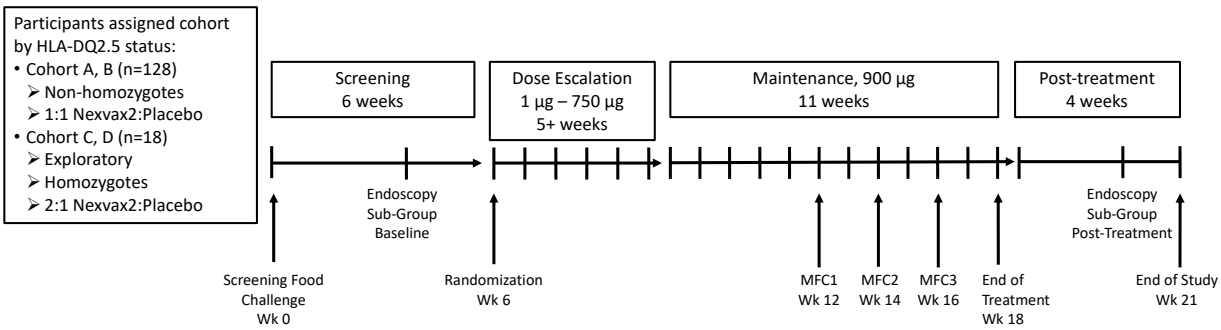

Figure S1. Study Design

References

1. Goel G, King T, Daveson AJ, et al. Epitope-specific immunotherapy targeting CD4-positive T cells in coeliac disease: two randomised, double-blind, placebo-controlled phase 1 studies. *Lancet Gastroenterol Hepatol* 2017; **2**(7): 479-93.