Epitope-specific immunotherapy targeting CD4+ T cells in celiac disease: evaluation in randomized, double-blind, placebo-controlled phase 1 studies

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SUMMARY

Background Gluten-free diet (GFD) is the only management available for celiac disease (CeD), a permanent immune intolerance to gluten. Nexvax2® is the first therapeutic vaccine designed to treat CeD. The adjuvant-free formulation of peptides is intended to engage and render gluten-specific CD4+ T cells unresponsive to further antigenic stimulation. We have assessed safety and pharmacodynamics of Nexvax2® in patients with CeD on GFD.

Methods In two randomized, double-blind, placebo-controlled, phase 1 studies at 12 community sites in Australia, New Zealand and the United States, we screened for HLA-DQ2·5+ CeD patients (aged 18–70 years) on GFD. The screening and post-treatment periods included either a crossover, placebo-controlled, oral gluten challenge (OGC) to mobilize and assess T cells responsive to Nexvax2 or, for the final cohort in each study, endoscopy and duodenal histology without OGC. Participants and study staff were masked to the gluten content of food provided for each interval of the OGCs. One of two sequences of active and placebo challenges was assigned (1:1) by central randomization using a simple block method. The sequence of challenges was active/placebo then active/placebo, or placebo/active then active/placebo for the OGCs in the screening and post-treatment periods, respectively. Participants with a negative interferon (IFN)-γ release assay (IGRA) to Nexvax2 peptides after the screening OGC, or Marsh score >1 were discontinued before dosing. There was temporal allocation of participants to sequential cohorts assessing multiple fixed intradermal doses of Nexvax2 (60µg, 90µg, or 150µg weekly in the 3-dose study; or 150µg, or 300µg two-times weekly in the 16-dose study) in 0.1 mL 0.9% sodium chloride. A maximum tolerated dose (MTD) was administered in the final biopsy cohort in each study. Participants within each cohort were assigned to receive Nexvax2 or placebo by central randomization (2:1, respectively) using simple block method in SAS software Version 9·2. Participants, investigators, and study staff were masked to the treatment assignment, except for the study pharmacist. The primary endpoint was the number and percentage of adverse events in the treatment period. Other safety outcomes included duodenal histology, gastrointestinal symptoms, plasma cytokines, and immune cell frequencies. The main pharmacodynamic endpoint was IGRA to Nexvax2 peptides. All participants who received Nexvax2 or placebo, the safety population, were included in an intention to treat analysis for the primary endpoint. Additional post hoc analyses were also performed. Both trials were completed and closed before data analysis. Trials were registered with Australian New Zealand Clinical Trials Registry, numbers ACTRN12612000355875 and ACTRN12613001331729.

Findings Participants were screened from November 28, 2012 to August 14, 2014, and August 3, 2012 to September 10, 2013, for the 3-dose and 16-dose studies respectively. Across both studies, 136 (80%) of 169 volunteers met initial eligibility criteria. After OGC, 62 (57%) of 108 participants were randomized, and after endoscopy 20 (71%) of 28 participants were randomized. The number of participants in the 3-dose study randomly allocated to placebo or active treatment arms were 3 and 9 in the first cohort assessing Nexvax2 60 µg, 4 and 9 in the second cohort assessing Nexvax2 90 µg, and 4 and 8 in the third cohort, and 3 and 3 in final (biopsy) cohort, which both assessed Nexvax2 150 µg. The number of participants in the 16-dose study randomly allocated to receive placebo or active treatment were 4 and 8 in the first cohort assessing Nexvax2 150 µg, 3 and 10 in the second cohort assessing Nexvax2 300 µg, and 7 and 7 in the final (biopsy) cohort which assessed Nexvax2 150 µg. The MTD for Nexvax2 was 150 µg

due to transient, acute gastrointestinal adverse events with onset at 2 to 5 h after initial doses of Nexvax2, similar to those caused by gluten ingestion. The total number of treatment emergent adverse events and percentage (%) of participants with at least one in the ascending dose cohorts of the 3-dose study were 15 (55%) in the 11 placebo-treated participants, 25 (56%) in 9 who received Nexvax2 60µg, 65 (78%) in 9 who received Nexvax2 90µg, and 16 (63%) in 8 who received Nexvax2 150µg, and 7 (100%) in the 3 placebo-treated participants and 1 (33%) in 3 participants randomized to Nexvax2 150µg in the biopsy cohort; in the 16-dose study, there were 13 (71%) in 7 placebo-treated participants, 21 (75%) 8 who received Nexvax2 150µg, 26 (100%) in 10 who received Nexvax2 300µg, and 24 (86%) in the 7 placebo-treated participants and 18 (71%) in 7 who received Nexvax2 150µg in the biopsy cohort. Vomiting, nausea and headache were the only treatment emergent adverse events that occurred in at least 5% of participants in either study. The total number of treatment emergent adverse events and percentage (%) of participants with at least one occurrence were: vomiting: 2 (22%) in 9 participants receiving Nexvax2 60µg, 5 (56%) in 9 receiving Nexvax2 90µg, 4 (50%) in 8 receiving Nexvax2 150µg in the 3-dose study; and 5 (63%) in 8 receiving Nexvax2 150µg, 4 (40%) in 10 receiving Nexvax2 300µg, 1 (14%) in 7 participants receiving placebo in the biopsy cohort of the 16-dose study; nausea: 1 (11%) with Nexvax2 60µg, 4 (44%) with Nexvax2 90µg, and 2 (25%) with Nexvax2 150µg in the 3-dose study, and none in the 16-dose study; headache: 4 (44%) with Nexvax2 90µg in the 3-dose study; and 3 (43%) for placebo in the 1st and 2nd cohorts, 3 (38%) with Nexvax2 150µg, 5 (50%) with Nexvax2 300µg, and 3 (43%) for placebo and 2 (29%) with Nexvax2 150µg in the biopsy cohort of the 16-dose study. Among Nexvax2-treated participants administered the MTD, the number of gastrointestinal treatment emergent adverse events were 8 in 4 (50%) of 8 participants in the 3rd cohort and none (0%) in 3 participants in the biopsy cohort of the 3-dose study, and 5 in 5 (63%) of 8 participants in the 1st cohort and 3 in 2 (29%) of 7 participants in the biopsy cohort of the 16-dose study. For the biopsy cohort of the 16-dose study, which tested the MTD, Nexvax2 was associated with 5 mild and 2 moderate drug-related adverse events in 4 (57%) of 7 participants compared to 5 mild adverse events in 3 (43%) of 7 placebo-treated participants. Comparing biopsies from screening and after the treatment period, median [interquartile range] villous height to crypt depth ratio in distal duodenal biopsies was not significantly different for Nexvax2 at the MTD with 3 doses over 15 days (2.04 [0.69] versus 2.49 [0·67], n=2), or 16 doses over 53 days (1·74 [0·54] versus 1·56 [0·58], n=7), and for placebo over 15 days (1.75 [0.62] versus 2.09 [0.71], n=3) or 16 doses over 53 days (2·10 [0·25] versus 1·92 [0·35], n=7). In those participants who completed the post-treatment OGC per protocol, IGRA was negative in 2 (22%) of 9 placebo-treated participants in the 3 dose study compared to 2 (33%) of 6 who received Nexvax2 60 µg, 5 (63%) of 8 who received Nexvax2 90 µg, and 6 (100%) of 6 who received Nexvax2 150 µg (p=0.007); and in 0 (0%) of 5 placebo-treated participants in the 16 dose study compared to 6 (75%) of 8 who received Nexvax2 150 µg (p=0.021).

Interpretation The maximum dose tolerated for Nexvax2 was established as 150µg for two-times weekly intradermal administration over 8 weeks. Administering Nexvax2 at the MTD for 8 weeks modified immune responsiveness to Nexvax2 peptides without deterioration in duodenal histology. The gastrointestinal symptoms that followed the first intradermal administration of Nexvax2 resembled those associated with oral gluten challenge. These findings support continued clinical development of Nexvax2 as a potential therapeutic vaccine for CeD.
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RESEARCH IN CONTEXT

Evidence before this study We searched for reviews of emerging treatments for celiac disease (CeD) published before Aug 24, 2016 in PubMed using the terms "Celiac disease" or "coeliac disease", and "non-dietary therapy", "immunotherapy", or "vaccine". We found 20 reviews of new treatments in development for CeD published since 2009, which confirmed that there are no therapeutics approved specifically for the treatment of CeD. We also searched Clinicaltrials.gov on Aug 24, 2016 for clinical trials using the terms "celiac disease therapy", "peptide-based immunotherapy", "peptide immunotherapy", "antigen-specific immunotherapy", "epitope-specific immunotherapy", and "specific immunotherapy". In total, we found clinical trials assessing 16 different agents for CeD, but Nexvax2 was the only antigen-specific therapeutic and none was at a stage of development more advanced than phase 2. These searches identified only 2 studies that assessed effects of immunogenic peptides for autoimmune diseases, both for type-1 diabetes (NCT01536431 and, not yet commenced, NCT02837094). The authors are also aware of two other recent clinical trials using immunogenic peptides for multiple sclerosis (NCT01097668 and NCT01973491).

Added value of this study The clinical effects and therapeutic potential of peptides recognized by disease-specific CD4+ T cells has not been systematically evaluated in clinical autoimmune diseases. Unlike other autoimmune diseases, the peptides responsible for the disease-specific CD4+ T-cell response to gluten associated with CeD are well characterized. Nexvax2 is the first antigen-specific immunotherapy under development for CeD and is an adjuvant-free solution of three peptides with immunodominant HLA-DQ2·5-restricted epitopes. The effects of systemically administered gluten peptides have not previously been tested. Intradermal administration of Nexvax2 in HLA-DQ2·5+ CeD participants initially caused gastrointestinal symptoms similar in timing and quality to those triggered by gluten ingestion. Adverse events after later administrations of Nexvax2 were no different from placebo. A maximum tolerated dose of Nexvax2 was established as 150 µg. There was no evidence of deterioration in duodenal histology following two-times weekly intradermal administrations over eight weeks with Nexvax2 150 µg. The recall immune response to gluten was modified in CeD participants receiving Nexvax2 consistent with T cells specific for epitopes in Nexvax2 were rendered unresponsive to further antigenic stimulation.

Implications of all the available evidence Repeated small, fixed doses of adjuvant-free peptides including immunodominant epitopes for gluten-specific CD4+ T cells are capable of modifying the recall immune response to gluten in CeD patients without causing duodenal injury. The clinical effects of systemically administering immunodominant epitopes for gluten-specific CD4+ T cells are at first similar to the gastrointestinal symptoms following gluten ingestion in CeD patients on GFD, but later doses are well tolerated with effects similar to placebo. These findings are consistent with Nexvax2 peptides engaging and rendering gluten-specific CD4+ T cells unresponsive to further antigenic stimulation. Further assessment and clinical development of antigen-specific immunotherapy for CeD using immunogenic gluten peptides is justified, and may inform the design, immune monitoring, and clinical development of this novel therapeutic class for autoimmune diseases.

INTRODUCTION

Celiac disease (CeD) is an autoimmune-like disease due to dietary gluten characterized by the presence of highly specific autoantibodies to transglutaminase 2 and damage of epithelial cells in the small intestine.[1] The community prevalence of CeD is about 1% in children and adults in many regions including Europe and North America.[2] Clinical investigation for CeD is usually prompted by digestive symptoms, associated co-morbidities such as iron deficiency, or screening family members of probands.[2] Abnormal CeD-specific serology and duodenal histology showing villous atrophy with crypt hyperplasia and intra-epithelial lymphocytosis support the diagnosis of CeD.[2] Currently, the only management for CeD is life-long gluten-free diet (GFD).[2] However, GFD is burdensome, and restrictive in social situations, resulting in reduced quality of life and, ultimately, non-adherence.[3,4] Consequently, GFD seldom results in complete clinical and histological recovery.

CeD has a remarkably strong association with certain Major Histocompatibility Complex (MHC) haplotypes that accounts for almost half of the total heritable risk of CeD.[5] About 90% of patients possess the MHC class II genes *HLA-DQA1*05* and *HLA-DQB1*02* that together encode the Human Leukocyte Antigen (HLA) heterodimer HLA-DQ2·5.[6] HLA-DQ2·5 homozygosity is associated with augmented T-cell activation by gluten peptides.[7]

MHC class II heterodimers serve a central role in antigen presentation and the induction and maintenance of acquired cellular immune responses.[8] The complex formed by short, antigen-derived peptides (epitopes) loaded into the binding groove of MHC class II molecules bind to the T cell antigen receptor (TCR) of CD4+ T cells, which results in highly specific antigen recognition and antigen-specific activation. CD4+ T cells specific for HLA-DQ2.5-restricted gluten peptides that secrete pro-inflammatory cytokines such as interferon(IFN)-γ can be isolated from intestinal tissue.[9] One week after commencing a 3-day gluten food challenge, these same CD4+ T cells circulate in blood at increased frequencies.[10] The amino acid sequence of immnodominant epitopes recognized by gluten-reactive CD4+ T cells are well established, and are highly consistent amongst HLA-DQ2.5+ CeD patients.[9-14]

Epitope-specific immunotherapy is a form of antigen-specific immunotherapy that uses peptides instead of whole antigen to target and modify CD4+ T cells.[15] In general, higher doses and longer duration of antigen-specific immunotherapy are most clinically effective.[16] Evidence supports that clinical benefit is related to disease-specific CD4+ T cells transitioning from being responsive to antigenic stimulation to a state of reversible functional unresponsiveness (anergy), induction of suppressive regulatory T cells, and eventually, over longer periods, to deletion and durable immune tolerance.[16]

Gluten itself is not suitable for a therapeutic vaccine because it is insoluble, requires deamidation for full immunogenicity, and some gluten peptides and contaminants have direct innate immune activity.[17,18] Nexvax2® is an adjuvant-free, particle-free solution of three, highly soluble, synthetic peptides with 15 or 16 amino acids (NPL001, NPL002, and NPL003) (appendix p 10). Nexvax2 has been designed and developed as an epitope-specific immunotherapy for HLA-DQ2.5+ CeD, which is further described in the appendix (pp 6-7). Nexvax2 encompasses at least five immunodominant epitopes that selectively bind to HLA-DQ2.5 and activate gluten-reactive

CD4+ T cells isolated from HLA-DQ2·5+ CeD patients (appendix p 10 and p 27).[14] These peptides include sequences recognized by anti-gliadin antibodies,[19] but are short enough to minimize the likelihood of complement activation by immune complex formation and antibody-mediated hypersensitivity.[20]

Our aim was to assess safety and pharmacodynamics of repeated intradermal administrations of Nexvax2 in regimens that could potentially modify gluten-specific immunity in HLA-DQ2·5+ CeD patients on GFD.

METHODS

Study design

Two separate randomized, placebo-controlled, double-blind, phase 1 studies were conducted. We tested Nexvax2 administered as 3 fixed doses at weekly intervals over 15 days ("3-dose study"), or 16 fixed doses at 3 or 4-day intervals over 53 days ("16-dose study") (appendix p 29). The studies were designed to establish a maximum tolerated dose (MTD) by testing incremental dose increases in a series of ascending dose cohorts. To mobilize gluten-specific T cells in blood and allow assessments of their responsiveness to epitopes in Nexvax2, the screening period and post-treatment period for ascending dose cohorts included a crossover, double-blind, placebo-controlled, oral gluten challenge (OGC). After the MTD was established, a "biopsy" cohort was enrolled in each study to test whether duodenal histology deteriorated following fixed dose administration of Nexvax2 at the MTD. Participants in biopsy cohorts did not have OGC before or after the treatment period to avoid the confounding effect of OGC on duodenal histology. Study sites are listed in the appendix (p 3). The studies were conducted concurrently with the 16-dose study recruiting exclusively from community sites in Australia and New Zealand, and the 3-dose study initially recruiting exclusively from community sites in the United States. After completion of the 16-dose study, participants for the second and later cohorts in the 3-dose study were also recruited sites in Australia and New Zealand. Approval was granted by local ethics committees listed in the appendix (pp 3-4). These studies were conducted according to the International Conference on Harmonisation harmonised tripartite guideline E6(R1): Good Clinical Practice. Research Assist (Bridgewater, New Jersey, USA) and CPR Pharma Services (Thebarton, South Australia, Australia) managed the studies.

Participants

The intended study population was patients who were HLA-DQ2·5+ with CeD on a GFD. Participants shown to be immunologically responsive to Nexvax2 assessed by whole IFN-γ release assay (IGRA) six days after commencing active gluten challenge during the screening period OGC were used to determine the MTD. Participants considered to be in histological remission at the screening endoscopy were used to assess the effects of Nexvax2 on duodenal histology. Inclusion criteria required that participants be aged 18 – 70 years, have had a diagnosis of CeD supported by histology and serology,[21] followed a GFD for over one year, and were HLA-DQ2·5+. Full eligibility criteria are provided in the appendix (pp 4-5). For randomization to treatment, participants in ascending dose cohorts required a positive IGRA to Nexvax2 peptides on screening day 6 or 13 that had returned to negative one week before dosing, and participants in biopsy cohorts were required to have duodenal histology at the

screening gastroscopy that was consistent with modified Marsh type 0 or 1 (no villous atrophy or crypt hyperplasia). All participants provided written informed consent before enrolment.

Randomization and masking

Trial sites assessed consecutive volunteers for eligibility. For each study, ascending dose cohorts were enrolled stepwise, beginning with the lowest dose level, and when they were complete the biopsy cohort was enrolled. Participants in ascending dose cohorts began the screening period with an OGC. The ordering of the gluten-containing (active) and gluten-free (placebo) challenges in the screening period was randomized with half the participants receiving active first, and the other half receiving placebo first. Allocation of participants to the order they received active and placebo gluten challenges in the screening period was by central randomization using simple block method with block size 200. Active was always before placebo gluten challenge in the post-treatment OGC. Cookies used in OGC were matched in physical appearance, consistency, and taste (appendix p 30). Participants were allocated active (Nexvax2) or placebo treatment by central randomization using a simple block method (block size 12) in a 2:1 schema for ascending dose cohorts, and 1:1 in biopsy cohorts. The appearance of syringes, drug product, and volume injected for active and placebo treatment administered in each trial were identical. Enrolment in cohorts and allocation of OGC sequence or treatment were not stratified for any additional factors. Replacements were allowed, and received the same treatment as the participant they replaced. The randomization mechanism for the study was deployed by sites completing a randomization request that was sent to the un-blinded statistician at CRC Pharma Services (Parsippany, NJ, USA) who provided the randomization number to the study site and notified the packager and distributor for OGC cookies (3-dose study: for sites in the USA: Research Assist, and for other sites: Pharmaceutical Packaging Professionals Pty Ltd., Port Melbourne, VIC, Australia; and for the 16-dose study cookies were provided direct to sites by the manufacturer, Shepherd Works, Boxhill North, Victoria, Australia), and for investigational product (Catalent Pharma Solutions; Allendale, NJ, USA). In the 3-dose study investigational product was provided in prefilled syringes with the site pharmacist being masked to treatment allocation. In the 16-dose study, the unmasked site pharmacist was responsible for diluting stock Nexvax2 9 mg/ml from labeled vials using USP 0.9% sodium chloride to the required concentration in 0.1 mL, or to draw USP 0.9% sodium chloride (0.1 mL) into a 1 mL syringes. The un-blinded statistician at CRC Pharma Services was provided the randomization schedule prepared by the central statistician, who was based at CPR Pharma Services, and had no responsibility for monitoring or data management, prepared randomization schedules using SAS software (SAS Institute Inc, Cary, North Carolina, USA) Version 9·2. The unmasked statistician at CRC Pharma, the packager and distributor of cookies and investigational product, and the pharmacist in the 16-dose study were the only other study personnel with copies of the randomization schedules. All study participants, care providers, data managers, sponsor personnel and study site personnel remained blinded to study treatment assignment until the analyses were completed.

Procedures

For ascending dose cohorts, the screening period began on day 1 with participants being provided nine cookies to eat 3 per day on days 1 to 3. When participants returned on day 8 they were provided another set of nine cookies for days 8 to 10. Cookies in each set either contained gluten (3 g per cookie), or were gluten-free (Shepherd Works). Patients with T cells responsive

to epitopes in Nexvax2 were identified using fresh blood and overnight IGRA.[22] IGRAs were performed with Nexvax2 peptides, and recall viral antigens on days 1, 6, 8, 13, and 29. Plasma cytokines and chemokines, and immune cell frequencies were also measured on day 1 (before cookies were eaten) and 13. If IGRA to Nexvax2 peptides became negative on day 29 the treatment period commenced one week later, but if IGRA remained positive it was repeated weekly until it became negative and the pre-treatment period was extended up to 49 days. For the biopsy cohorts, the screening period was 35 days with a gastroscopy between days 15 and 28; modified Marsh type was determined in duplicate biopsies collected from the bulb, 1st, 2nd, and 3rd parts of the duodenum to assess eligibility.

Three, weekly intradermal injections of placebo or Nexvax2 60μg, 90μg, or 150μg in 0·1 mL were administered in the 3-dose study. In the 16-dose study, 16 two-times weekly doses of placebo or Nexvax2 150μg, or 300μg in 0·1 mL were administered. Injections administered by study staff on site alternated between the supra-deltoid regions on each arm. When at least six participants who received active treatment had completed at least two weeks of treatment, and if the Dose Escalation Committee (DEC) considered that masked clinical and laboratory pathology safety assessments showed an acceptable safety profile according to predefined criteria described in the appendix (p 5), dosage was increased in the next cohort. For additional consideration of safety data and the decision to dose-escalate or discontinue dosing the DEC could consult the independent safety monitor, a designated member of the independent data safety monitoring board (DSMB), or the full DSMB, which could have access to unmasked safety data. MTD was the highest dose reviewed and approved by the DEC.

Safety assessments included the incidence, severity, and dose relation of adverse events. To elicit adverse events, at every study visit, participants were asked, "Have you had any health problems since the previous visit or when you were last asked?" and "Have you had any new symptoms?" The blinded local site investigators were responsible for reporting and coding adverse events using MedDRA v15·0, and grading their severity and causality according to "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials".[23] Other safety assessments included changes in laboratory haematology and chemistry, and urinalysis variables, self-reported weekly gastrointestinal symptom rating scale (GSRS),[24] and self-assessed daily GSRS dimensions of pain (Q1), hunger pains (Q4), nausea (Q5), rumbling (Q6), bloating Q7), and diarrhoea (Q11) (except on days when the GSRS was recorded). Vital signs, physical examination, 12-lead electrocardiograph, cytokine and chemokine measurements, and immune cell frequencies were also safety assessments. Plasma cytokines and chemokines, immune cell frequencies, and IGRA were measured in the treatment period before the first and last doses, on day 8, and in the 16-dose study on day 25 and 39. Plasma cytokines and chemokines were also assessed 6h after the first and last doses. Pharmacokinetics was assessed up to 6h after the first and last dose.

In the 4-week post-treatment period, ascending dose cohorts had a further OGC, and biopsy cohorts had a gastroscopy with duodenal biopsies and quantitative histology within two weeks. IGRA, plasma cytokines and chemokines, and immune cell frequencies were assessed on day 13; IGRA was also performed on the 1st, 6th, and 8th days, and at end of study (EOS).

Serum IgG and IgA specific for the pool of Nexvax2 peptides were measured at screening, treatment period days 1 and 8, and also 25 and 39 in the 16-dose study, and post-treatment on day 1 and at EOS.

Laboratory assays are described in the appendix (pp 6-8).

Post hoc assessments included complement levels in plasma collected for cytokine assessments on treatment days 1, 8 and 53 pre-dose as well as 6h post-dose on day 1 and 53 in both cohorts receiving Nexvax2 150μg of the 16-dose study; and CeD serology in sera collected for anti-Nexvax2 antibody assessments. Post-treatment "responders" to Nexvax2 were defined post hoc as having Nexvax2-specific IGRA positive six or eight days after commencing the gluten segment in OGC, and "non-responders" as Nexvax2-specific IGRA negative six and eight days after commencing gluten only when all nine gluten cookies had been consumed.

Outcomes

The primary endpoint in each study was centrally assessed as the number and percentage of adverse events during the treatment period. Secondary endpoints included other safety and tolerability assessments during the treatment and post-treatment periods, and serum anti-Nexvax2 antibodies. Pharmacokinetics endpoints were maximal concentrations and area under the curve for NPL001, NPL002, and NLP003. Quantitative duodenal histology was an exploratory safety endpoint. Nexvax2-specific IGRA was defined as the main pharmacodynamic endpoint. Post hoc analyses addressed IGRA to recall CMV-EBV-inFluenza (CEF) epitopes; gastrointestinal symptoms and IGRA during the screening OGC.

Statistical analysis

The sample size was pragmatic to assess the safety and tolerability of Nexvax2, while minimizing unnecessary participant exposure. All participants who received Nexvax2 or placebo, the safety population, were included in an intention to treat analysis for the primary endpoint. Additional post hoc analyses were also performed. Both trials were completed and closed before data analysis. No formal per protocol statistical analyses were planned, but post hoc analyses were performed and are described in the appendix (p 8). To address the confounding effects of reduced gluten exposure in the post-treatment OGC, an algorithm was developed post hoc to define the populations for post-treatment pharmacodynamic analysis (appendix p 31). Statistical tests were two-sided with a significance level of $p \leq 0{\cdot}05$. FDR-adjusted p-values, estimated using Benjamini-Hochberg method, were used where indicated to account for multiple hypothesis testing. All analysis was done using MATLAB software. Data were collected by the investigators, managed by CPR, and analysed by Prometrika (Cambridge, Massachusetts, USA) and the academic co-authors. Trials were registered with the Australian New Zealand Clinical Trials Registry, numbers ACTRN12612000355875, and ACTRN12613001331729.

Role of funding source

The funder of the study was involved in the study design, data collection, data analysis, data interpretation, and the writing of this report. GG, LJW, RJX, and RPA had full access to all the data in the study. RPA had final responsibility for the decision to submit for publication.

RESULTS

Between 28 November 2012 and 14 August 2014, 102 volunteers were enrolled in the 3-dose study. 21 (21%) of these 102 volunteers did not meet eligibility criteria on screening day 1. Subsequently, 69 (68%) of the original 102 volunteers were screened for ascending dose cohorts and 12 (12%) for the biopsy cohort (Figure 1A). Ultimately, 37 (54%) of 69 participants screened for ascending dose cohorts continued to treatment randomization after OGC. The 32 (46%) of 69 participants screened for ascending dose cohorts who did not continue included 17 (25%) that were IGRA negative, and 9 (13%) with IGRA persisting positive. 6 (50%) of 12 participants screened for the biopsy cohort continued to randomization following gastroscopy after 4 (33%) were excluded with villous atrophy and 2 (17%) withdrew. In the first dose cohort, nine participants were randomly allocated to receive Nexvax2 at 60 µg and three to placebo; in the second dose cohort, nine participants were randomly allocated to receive Nexvax2 at 90 µg and four to placebo; in the third dose cohort, eight participants were randomly allocated to receive Nexvax2 at 150 µg and four to placebo; and in the final (biopsy) cohort three participants were randomly allocated to receive Nexvax2 at 150 µg and three to placebo. Each placebo-treated participant received three doses, but the Nexvax2-treated participants included two who withdrew during dosing from the 60µg and 90µg arms, and another was discontinued due to elevated transaminases pre-dose on the first day of treatment. All participants who completed dosing commenced OGC one day after last dose, or had a second gastroscopy. All 43 participants randomized to treatment were included in the primary endpoint analysis of safety. Table 1 lists the baseline characteristics of the study participants randomized to treatment; 70% were women, mean age was 45 years, and on average CeD had been diagnosed 8 years previously.

Between 3 August 2012 and 10 September 2013, 67 volunteers were enrolled in the 16-dose study. 12 (18%) of the original 67 volunteers did not meet eligibility criteria on screening day 1. Subsequently, 39 (58%) of the original 67 volunteers were screened for ascending dose cohorts and 16 (24%) for the biopsy cohort (Figure 1B). Ultimately, 25 (64%) of 39 participants screened for ascending dose cohorts continued to treatment randomization after OGC. 7 (18%) of the participants screened for ascending dose cohorts were discontinued before treatment randomization because IGRA to Nexvax2 peptides was negative. 14 (88%) of 16 participants continued to randomization after gastroscopy in the biopsy cohort after 2 (12%) were excluded with villous atrophy. In the first dose cohort, eight participants were randomly allocated to receive Nexvax2 at 150 µg and four to placebo; in the second dose cohort, ten participants were randomly allocated to receive Nexvax2 at 300 µg and three to placebo; and in the final (biopsy) cohort seven participants were randomly allocated to receive Nexvax2 at 150 µg and seven to placebo. All participants in the first and final cohorts received at least 15 of 16 doses, and then commenced OGC one day after last dose or had a second gastroscopy. In the second cohort, five participants received no more than 3 doses of Nexvax2 300µg, and five participants received between 4 and 16 doses of Nexvax2 and then commenced OGC within 3 days of the last dose. Participants in the second cohort who received placebo had only 5, 10 or 15 doses and then commenced OGC within 3 days of the last dose. All 39 participants randomized to treatment in the 16-dose study were included in the primary endpoint analysis of safety. Baseline characteristics of the study participants randomized to treatment were similar to the 3-dose study

(Table 1); 79% were women, mean age was 45 years, and on average CeD had been diagnosed 7 years previously.

The number, percentage, and severity of adverse events collected during the treatment period for each cohort and in each study are shown in appendix (pp 11-12). The organ systems affected by adverse events are shown in Figure 2 and appendix (pp 13-15). The timing of adverse events in relationship to dosing, and dose number are shown in Figure 2. Adverse events of at least moderate severity are shown in appendix (pp 16-18), and those that affected the gastrointestinal system are summarized in Table 2. Overall, participants in either study who received Nexvax2 were more likely to experience adverse events after the first dose than placebo-treated participants (p=0·0085; Fisher's Exact test) (Figure 2). In ascending dose cohorts whose screening period always included OGC, the first dose of Nexvax2 was frequently followed by adverse events affecting the gastrointestinal system within 2 to 5 hours (Figure 2); nausea with or without vomiting, abdominal pain, and/or diarrhea were common (Table 2). Adverse events and symptoms with Nexvax2 were reduced with subsequent doses, and were no different from placebo by the third week of dosing (Figure 2A, E). Gastrointestinal and systemic adverse events graded severe included nausea, abdominal pain, vomiting, shivering, clammy skin, and or rigors occurred 2 to 5 hours after the first or second dose of Nexvax2 (appendix pp 16-18). All 7 participants experiencing gastrointestinal and/or systemic adverse events graded severe, including one graded serious – abdominal pain) were in ascending dose cohorts with their screening period including OGC. Five (71%) of the seven participants experiencing these post-dose gastrointestinal adverse events graded severe were homozygous for *HLA-DQA1*05* and *HLA-DQB1*02*, significantly more than among other participants in ascending dose cohorts (9 (26%) of 35 participants, p=0.0313; Fisher's Exact test) (Table 2 and appendix pp 16-18). All five (100%) of the five participants with an adverse event graded severe or serious after the first dose of Nexvax2 were homozygotes for *HLA-DQA1*05* and *HLA-DQB1*02*. In the 3-dose study, gastrointestinal and/or systemic adverse events graded severe or serious occurred after the first dose in 1 (11%) of 9 participants receiving of Nexvax2 60 μg, in 3 (33%) of 9 participants receiving Nexvax2 90 μg (in 2 after the first dose, and in one after both the second and third doses), and after the second dose in 1 (11%) of 8 participants receiving Nexvax2 150 μg. In the 16-dose study, gastrointestinal adverse events were graded severe in one (13%) of 8 participants after the first dose of Nexvax2 150 μg, and as serious (abdominal pain associated with vomiting) in 1 (10%) of 10 participants after the first dose of Nexvax2 300 μg. Dosing was discontinued in the second cohort of the 16-dose study on the recommendation of the DSMB following unmasked review of safety data summarized in appendix (pp 16-18). According to the dose escalation criteria (appendix pp 5-6), theMTD of 150 μg was established for Nexvax2 administered at intervals of 3 to 4 days by intradermal injection.

Consistent with adverse events, weekly GSRS scores significantly increased in Nexvax2-treated participants for the first week of twice-weekly dosing (appendix p 19), and there was a trend for gastrointestinal symptoms to increase on each dosing day for Nexvax2-treated participants over the first two weeks of the treatment period in both studies (Figure 3A-F). However, nausea was the only symptom to increase and reach statistical significance on the first day of dosing in any cohort (Figure 3A; p=0·015; Wilcoxon rank sum test). Overall, the clinical effects of administering the first dose of Nexvax2 resembled the symptoms reported by participants during

the screening period when they consumed gluten and experienced significantly increased abdominal pain, nausea, rumbling, bloating, and diarrhea (appendix p 20).

Clinical and laboratory safety assessments including circulating lymphocyte subsets (appendix pp 21-23), and anti-Nexvax2 IgG and IgA (appendix p 32), showed no significant changes over the treatment period. Seroconversion for antibodies specific for transglutaminase 2 and deamidated gliadin peptide was not observed from screening to end of study (appendix p 24). Comparing biopsies from screening and after the treatment period, median [interquartile range] villous height to crypt depth ratio in distal duodenal biopsies was not significantly different for Nexvax2 at the MTD with 3 doses over 15 days (2.04 [0.69] versus 2.49 [0·67], n=2), or 16 doses over 53 days (1·74 [0·54] versus 1·56 [0·58], n=7), and for placebo over 15 days (1.75 [0.62] versus 2.09 [0.71], n=3) or 16 doses over 53 days (2·10 [0·25] versus 1·92 [0·35], n=7). Villous height to crypt depth ratio in proximal duodenal biopsies, density of intra-epithelial lymphocytes, and modified Marsh scores were also not different in biopsies collected before and after dosing with Nexvax2 150µg or placebo weekly over 15 days or two-times weekly over 53 weeks (appendix p 25). Complement levels were stable during the treatment period (appendix p 26). Pre-dose plasma cytokines and chemokines were stable over the study period; post-dose alterations in plasma cytokines and chemokines are reported elsewhere (publication in preparation).

Plasma concentrations of NPL001, NPL002, and NPL003 were above levels of detection (0·05 nM, 0·1 nM, and 0·4 nM, respectively) from ten minutes up to two hours after the first and final administrations of Nexvax2 in both studies, but were at levels below the limits of quantitation (2·6 nM, 5·5 nM, and 5·3 nM, respectively) (appendix p 33).

Unlike gluten challenge during the screening period, Nexvax2 administration did not cause the whole blood IGRA for Nexvax2 peptides to become positive (Figure 4). In contrast, IGRA responses to recall epitopes were present and stable throughout the study (appendix p 34).

Aggregate daily symptoms score during the post-treatment OGC was significantly worse than pre-treatment in placebo-treated participants (p=0·0232, Wilcoxon Signed-Rank Test). Almost half the placebo-treated participants did not consume all gluten-containing cookies during the post-treatment gluten challenge, which was significantly less than Nexvax2-treated participants across both studies (10/18 vs 31/33; p=0·0019, Fisher's Exact Test, Table 3). Post hoc analysis of Nexvax2-specific IGRA showed that participants treated with Nexvax2 150µg in the 3-dose or 16-dose study were mostly IGRA negative to Nexvax2 peptides when they completed the gluten challenge, but almost all placebo-treated participants who completed gluten challenge were positive (Table 3; 3-dose-150µg: 6/6 versus 2/9, p=0·007; 16-dose-150µg: 6/8 versus 0/5, p=0·021, Fisher's Exact Test).

DISCUSSION

CeD represents a unique condition amongst the autoimmune diseases since the main components in the etiology and pathogenesis have been recognized: the MHC Class II haplotype contributes almost half of the genetic susceptibility,[5] and the hierarchy of epitopes recognized by CD4+ T cells responding to the trigger antigen are well characterized.[13] In addition, the causative antigen,

gluten, can be reintroduced to patients to assess immune responsiveness and its effects on the target organ.[15] For these reasons, CeD is exceptionally well positioned for the development and testing of epitope-specific immunotherapy, complimenting the limited clinical experience of this novel class of antigen-specific immunotherapy in allergy and autoimmunity, and translating insights from preclinical models.[25]

The phase 1 studies of Nexvax2 are the first to assess the clinical and immunological effects of systemically administered peptides implicated in the adaptive immune response in CeD. The target for Nexvax2 is the HLA-DQ2.5-epitope-TCR complex linking the surfaces of antigen presenting cells and gluten-reactive CD4+ T cells.[26] *In vitro*, the component peptides in Nexvax2 bind selectively to HLA-DQ2.5, but not other CeD-associated HLA-DQ heterodimers. The peptides also activate CD4+ T cell clones from CeD patients that are specific for HLA-DQ2.5-restricted epitopes represented in Nexvax2. *In vivo*, Nexvax2 engagement with its predicted target was supported by observing that whole blood IGRA for Nexvax2 peptides was converted from positive after pre-treatment OGC to negative after post-treatment OGC in most participants receiving Nexvax2 150μg. Furthermore, to our knowledge, systemic administration of epitopes for CD4+ T cells implicated in an autoimmune or allergic disease has not previously been associated with digestive symptoms, which suggests this may a specific effect of gluten epitopes. Additional indirect evidence of target engagement was supported by observing that the first dose of Nexvax2 is followed by digestive symptoms, which are similar in timing and quality to those associated with gluten exposure.

Despite the first administration of Nexvax2 being followed by symptoms typical of gluten exposure in CeD, repeated dosing over 8 weeks did not affect duodenal histology. Duodenal mucosal histology is slow to recover after institution of gluten-free diet, and would not be expected to show improvement over the duration of the treatment periods in these phase 1 studies. In contrast, daily gluten ingestion can cause symptoms on the first day similar to those after the first dose of Nexvax2 150 μg, and result in pronounced mucosal damage after one week.[27] There was a significant reduction in symptoms without changes in pharmacokinetics or anti-Nexvax2 antibodies after the final doses of Nexvax2 in both studies. This was consistent with target T cells becoming functionally unresponsive to antigenic stimulation. This interpretation was supported by whole blood IGRA for Nexvax2 peptides frequently being negative after gluten challenge in the post-treatment period in participants administered Nexvax2. Direct visualization of peripheral blood and intestinal gluten-specific T cells assessed by flow cytometry using MHC-peptide complexes will be required in future studies to address whether CD4+ T cells specific for gluten, and in particular Nexvax2, express surface markers consistent with anergy or regulatory function following treatment with Nexvax2.[11]

These phase 1 studies were not designed to address the efficacy of Nexvax2 in CeD. Clinically relevant endpoints such as patient reported outcome measurements and quantitative histology after longer periods of treatment will be required to assess efficacy. In this study, we had intended to determine the MTD for Nexvax2 in participants who mobilized CD4+ T cells responsive to Nexvax2. However, a potential limitation to the current studies was the high number of participants ineligible for dosing. The most common reason for exclusion from ascending dose cohorts was negative IGRA to Nexvax2 peptides after gluten challenge in the screening period. Because virtually all HLA-DQ2.5+ CeD patients harbor CD4+ T cells specific

for epitopes in Nexvax2,[9-14] the most likely explanation for failure to mobilize Nexvax2-specific CD4+ T cells was recent, inadvertent gluten ingestion.[28] Exclusion of participants with significant mucosal injury from biopsy cohorts was also most likely due to inadvertent non-adherence to GFD.[29]

Together these studies support the safety, tolerability, and relevant bioactivity of Nexvax2. Gradual escalation up to the maintenance dose of a peptide immunotherapy may be tolerated better than fixed dose regimens.[30] A separate study is addressing whether symptoms associated with initial and maximal doses of Nexvax2 are overcome by gradual dose escalation (ClinicalTrials.gov NCT02528799). These studies provide the basis for future clinical trials to test whether Nexvax2 protects against the damaging effects of gluten in HLA-DQ2·5+ CeD.

Contributors RPA, LW and PHG designed the studies. TK, AJD, JAM, JK, RK, GB, RF, CFB, RE, TPK, and PBM served as trial site principal investigators. JAT-D was medical monitor and performed immunogenicity testing and immune monitoring along with AG. JT, AP and MM performed histological analysis. JLD, KEG, and SW executed immune monitoring assays. JS performed supplementary MHC Class II peptide binding assays in the lab of AS. Data integration and analysis was performed by GG, RJX, and RPA. Tables and figures were prepared by GG and RPA. BJ and LMS assisted in interpretation of results. GG, BJ and RPA wrote the manuscript. All authors reviewed and approved the manuscript, tables and figures. The authors made the decision to submit the manuscript for publication and vouch for the accuracy of the data and analyses and for the fidelity of this report to the trial protocol. RPA had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests JMA is a consultant to Abbvie, Abbott, Ferring, Janssen, MSD, Hospira, Pfizer, Takeda, and Shire. JAT-D has received honoraria from ImmusanT, Inc. as a consultant and medical monitor on the clinical trials. JAT-D and RPA are co-inventors of patents, owned or licensed by ImmusanT, Inc. and RPA has also received royalties from those licensed to ImmusanT, Inc., covering the composition of Nexvax2, and utilization of gluten-derived T cell epitopes for use in therapeutics. RPA has additional patents covering the uses of epitopes in diagnostics, food tests and non-toxic cereals, all of which are owned or licensed by ImmusanT, Inc. MM, RJX, LMS and BJ are members of the Scientific Advisory Board of ImmusanT, Inc. MM is a scientific advisor to Celimmune, LLC, and holds a patent on methods and means for detecting gluten-induced diseases that is owned by Tampere University and licensed to Labsystems Diagnostics, Finland. LMS receives honoraria for scientific consulting provided to ImmuanT, Inc., Regeneron, Glenmark, and Celgene, and holds a patent on methods for detection of gluten-specific T-cells. BJ serves as a scientific advisor to Bioniz and Celimmune. LJW, RPA, KEG, SW and JLD are employees of ImmusanT, Inc. PHG is a former employee of ImmusanT, Inc. The other authors declared no conflicts of interest.

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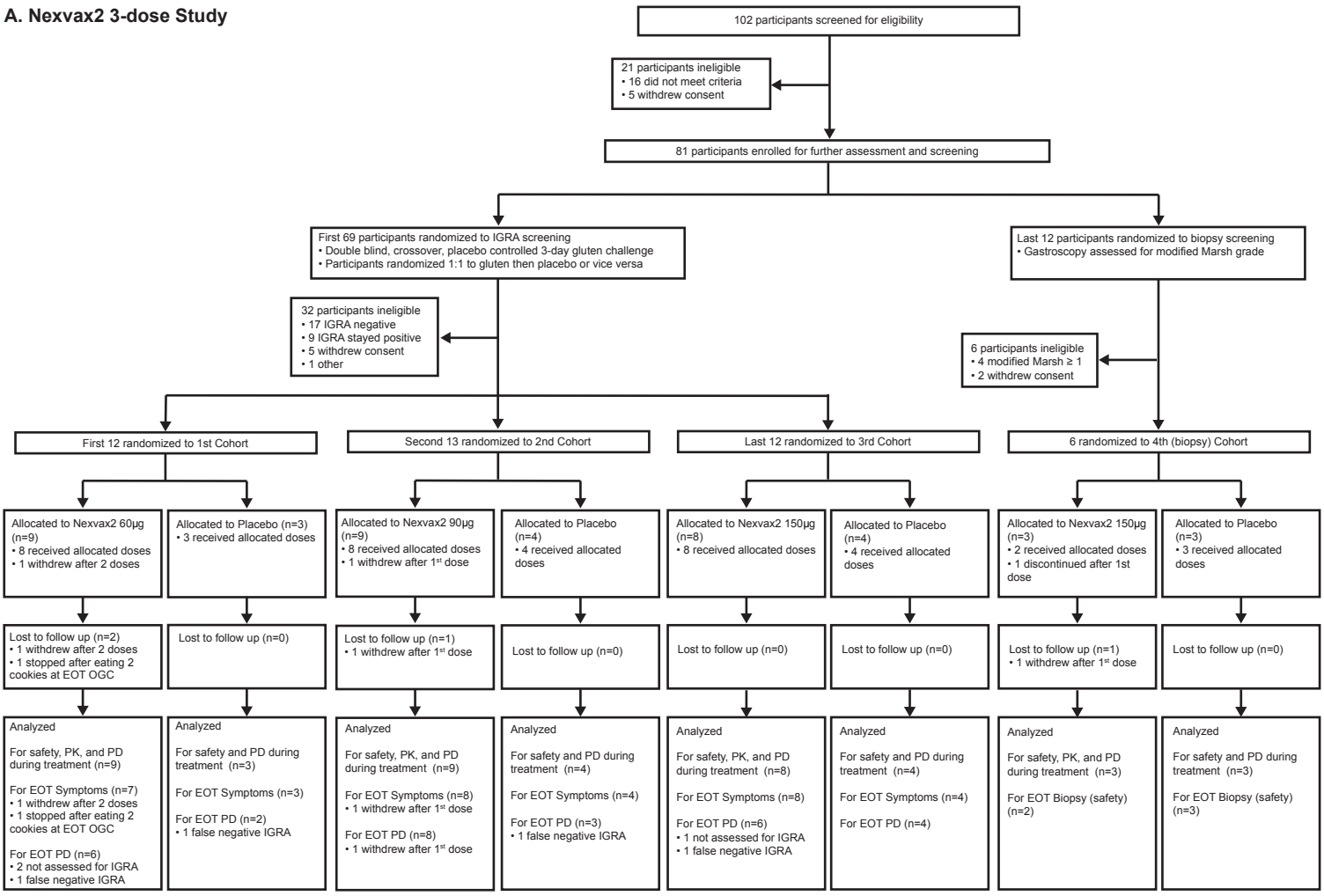
FIGURE LEGENDS

Figure 1: Trial profiles.

Figure 2: Treatment emergent adverse events (TEAE) after each dose of Nexvax2 or placebo. Number of participants experiencing TEAEs of maximal severity grading mild, moderate, severe, serious or none are shown in **A, C, E, G, I, K and M**, and total number of TEAEs classified by organ system after each dose are shown in **B, D, F, H, J, L, and N** (Nervous system disorder was predominantly "Headache"; Gastrointestinal disorder was frequently "Vomiting", "Nausea", "Abdominal Pain", or "Diarrhea"; General disorders included "Injection Site Pain" and "Fatigue"). More participants experienced TEAEs after the first dose of Nexvax2 **(A, C, G, and K)** (N=54) than participants receiving placebo **(E, I, and M)** (N=28) (p=0·0085; Fisher's Exact test), but the only individual dose level that reached significance compared to its matched placebo was Nexvax2 300 μg in 2nd Cohort of 16-dose study **(C and E)** (p=0·0498; Fisher's Exact test). Most TEAEs after the first dose of Nexvax2 affected the gastrointestinal system. The number of participants with any TEAE, and the frequency of TEAEs after later doses of Nexvax2 were not significantly different from placebo.

Figure 3: Gastrointestinal symptoms. Participants scored six items in the Gastrointestinal Symptoms Rating Scale (GSRS) from 1 (no discomfort at all) to 6 (very severe discomfort) every day except the last day of each week during the 16-dose **(A-D)**, and 3-dose studies **(E and F)**. In ascending dose cohorts **(A, C, E and F)**, 3-day gluten challenge (GC) corresponds to Screening days 1 to 3, and placebo challenge (PC) corresponds to Screening days 8 to 10; the biopsy cohorts did not have a gluten challenge **(B and D)**. The sum of six symptom scores increased when Nexvax2 was first administered (Treatment day 1) and reached statistical significance in participants receiving Nexvax2 150μg **(A)** compared to placebo **(C)** in ascending dose cohorts of the 16-dose study (P=0·015; Wilcoxon rank sum test).

Figure 4: Activation of T cells. The presence and functional responsiveness of circulating T cells specific for epitopes in Nexvax2 was tested by *ex vivo* whole blood interferon(IFN)-γ release assay (IGRA). Fold increase in IFNγ release (stimulation index) stimulated by Nexvax2 peptides compared to medium is shown for participants in the 16-dose study receiving Nexvax2 150μg in the 1st cohort (n=8) **(A)**, and in the 3rd (biopsy) cohort that did not have screening gluten challenge (n=7) **(B)**; median with interquartile range are shown.



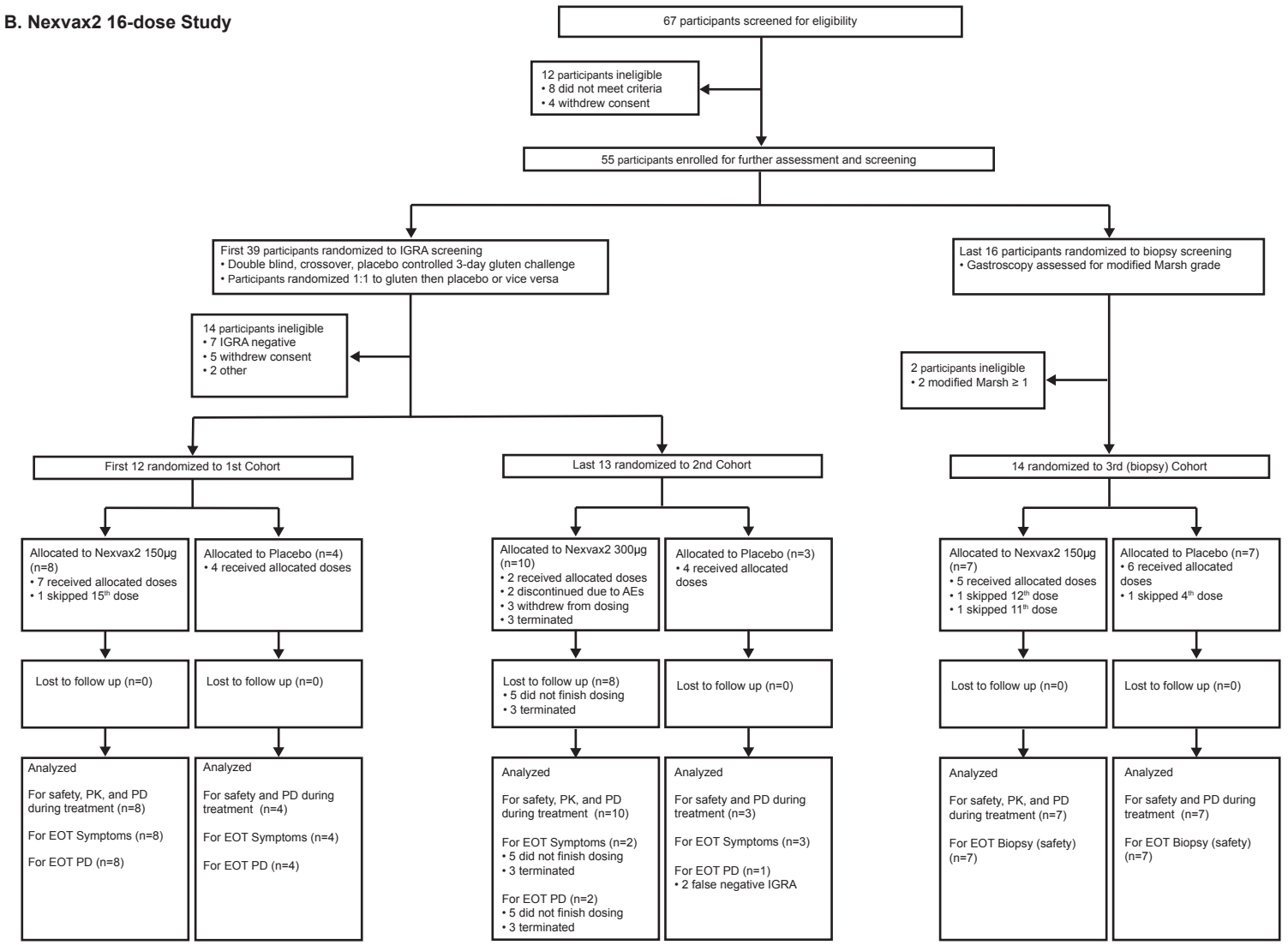


Figure 1: Trial Profiles

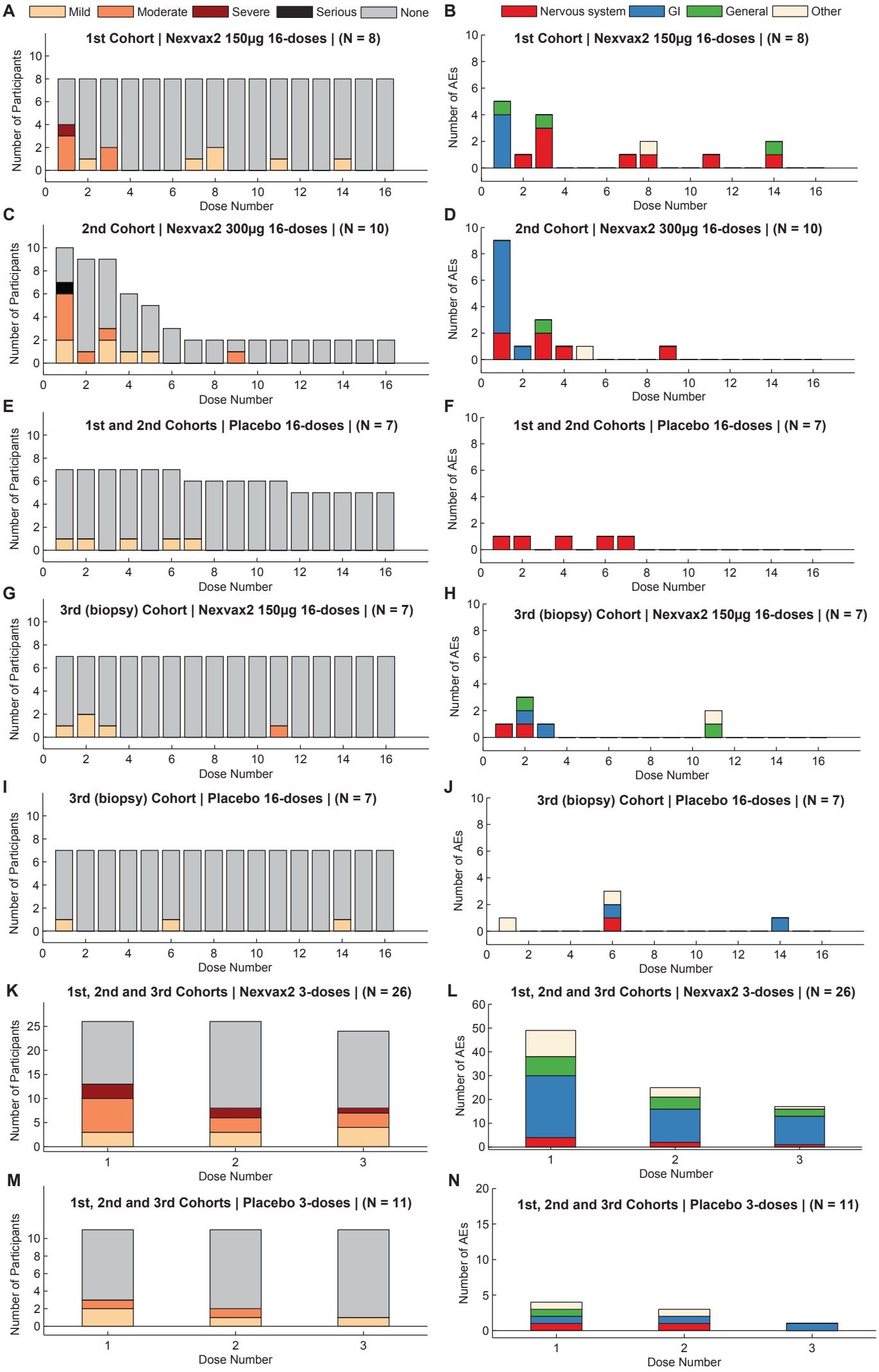


Figure 2: Treatment emergent adverse events (TEAE) after each dose of Nexvax2 or placebo

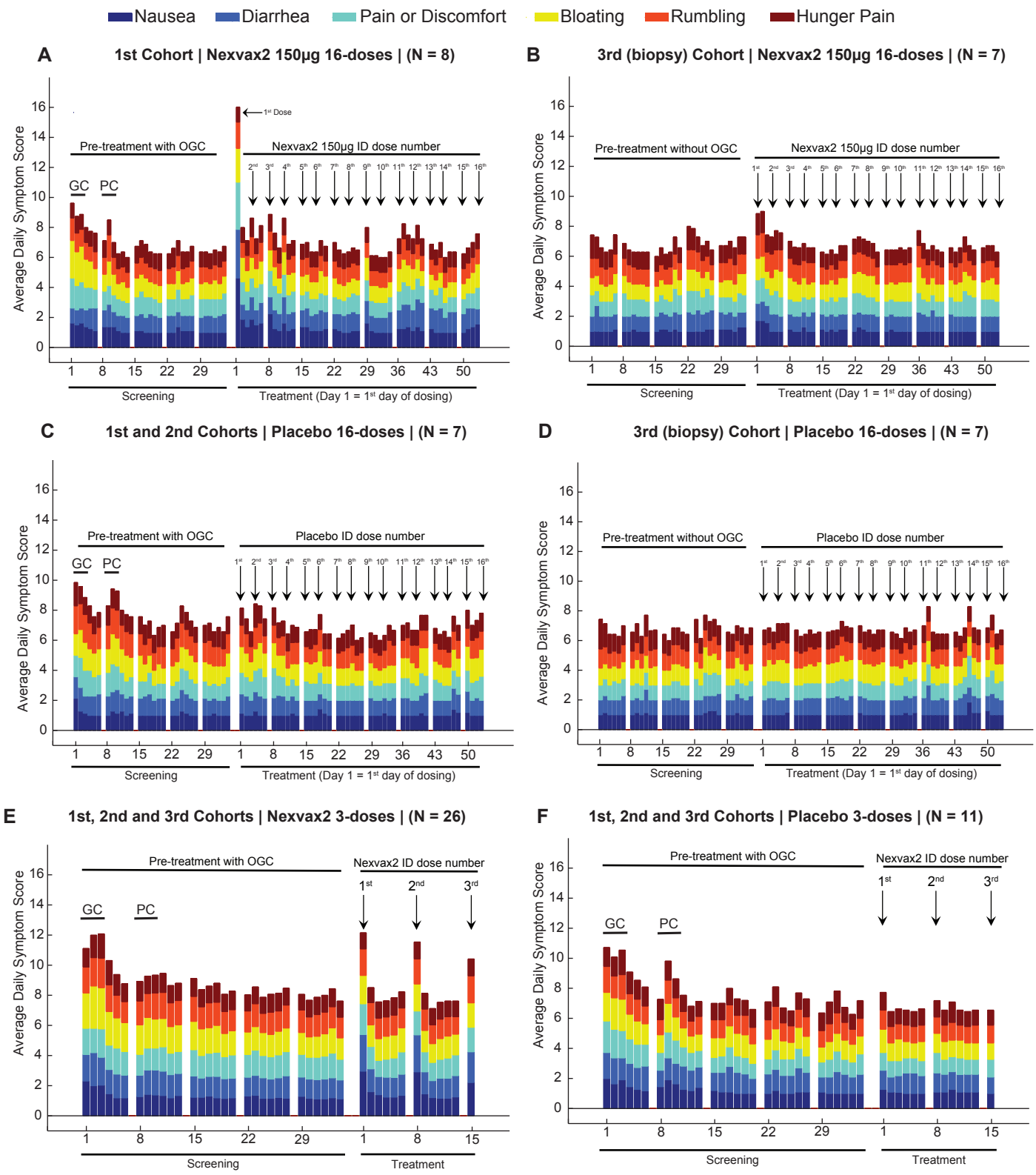


Figure 3: Gastrointestinal symptoms

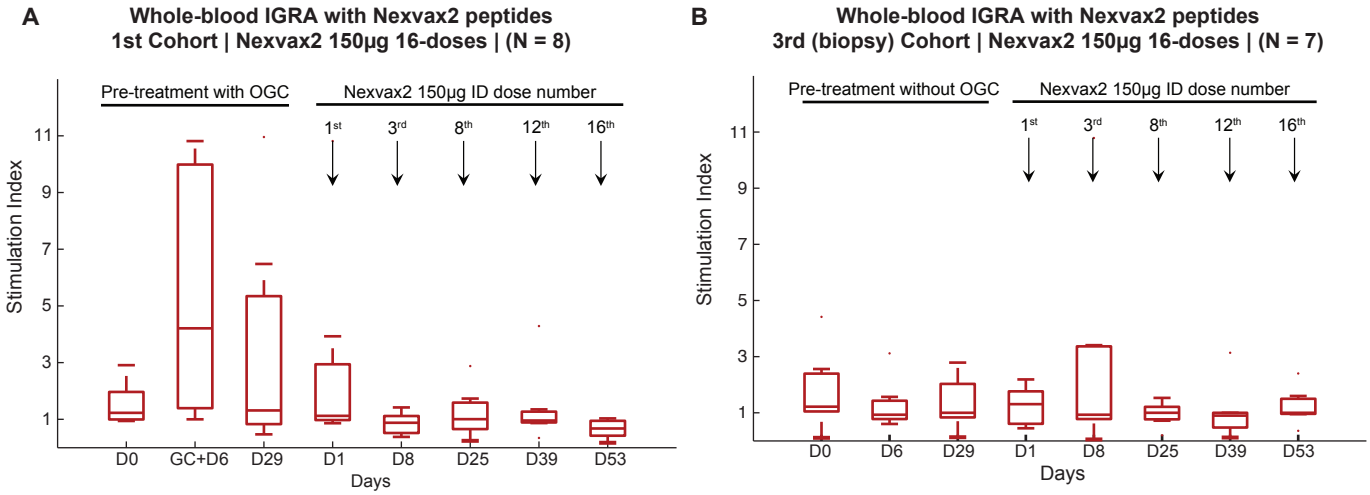


Figure 4: Activation of T cells

Table 1: Baseline characteristics of participants

3-dose study							
Cohort/s Treatment	**1st, 2nd & 3rd Placebo**	**1st Nexvax2 60 µg**	**2nd Nexvax2 90 µg**	**3rd Nexvax2 150 µg**	**4th (biopsy) Placebo**	**4th (biopsy) Nexvax2 150 µg**	**All Participants Dosed**
N	(n=11)	(n=9)	(n=9)	(n=8)	(n=3)	(n=3)	(n=43)
Age, years	44·2 (14·4)	48·8 (13·5)	47·6 (12·3)	47·9 (17·4)	34·0 (16·8)	27·3 (11·4)	44·7 (14·8)
Men	2 (18%)	3 (33%)	3 (33%)	3 (38%)	0	2 (67%)	13 (30%)
Women	9 (82%)	6 (67%)	6 (67%)	5 (62%)	3 (100%)	1 (33%)	30 (70%)
Race, White	11 (100%)	9 (100%)	9 (100%)	7 (87%)	3 (100%)	3 (100%)	42 (98%)
Race, Arab	0	0	0	1 (13%)	0	0	1 (2%)
Age at CeD diagnosis, years	38·5 (13·7)	42·7 (12·8)	35·5 (15·5)	34·0 (19·1)	28·9 (16·8)	25·9 (11·4)	36·4 (15·0)
Body mass, kg	77·5 (14·0)	94·9 (17·8)	72·5 (15·5)	79·6 (18·6)	67·3 (4·9)	96·0 (24·3)	81·1 (18·2)
BMI, kg/m^2	27·1 (3·9)	32·7 (4·6)	25·7 (4·8)	27·2 (3·3)	24·0 (3·0)	27·9 (3·8)	27·8 (4·7)
Homozygous HLA-DQ2·5	5 (45%)	4 (44%)	3 (33%)	2 (25%)	0	1 (33%)	15 (35%)

16-dose study							
Cohort Treatment	**1st Placebo**	**1st Nexvax2 150 µg**	**2nd Placebo**	**2nd Nexvax2 300 µg**	**3rd (biopsy) Placebo**	**3rd (biopsy) Nexvax2 150 µg**	**All Participants Dosed**
N	(n=4)	(n=8)	(n=3)	(n=10)	(n=7)	(n=7)	(n=39)
Age, years	47·0 (9·8)	52·0 (11·9)	39·0 (23·3)	50·0 (10·1)	34·6 (15·1)	42·6 (5·4)	45·2 (13·0)
Men	1 (25%)	1 (13%	1 (33%)	3 (30%)	2 (29%)	2 (29%)	10 (26%)
Women	3 (75%)	7 (87%)	2 (67%)	7 (70%)	5 (71%)	5 (71%)	29 (79%)
Race, white	4 (100%)	8 (100%)	3 (100%)	10 (100%)	7 (100%)	7 (100%)	39 (100%)
Age at CeD diagnosis, years	42·3 (9·9)	43·4 (12·7)	31·1 (17·4)	42·0 (10·8)	29·0 (11·8)	37·6 (5·4)	38·6 (11·5)
Body mass, kg	63·1 (17·7)	70·7 (11·2)	66·8 (12·4)	85·3 (13·0)	68·5 (11·8)	74·4 (11·6)	73·6 (14·1)
BMI, kg/m^2	22·8 (3·2)	25·3 (4·3)	22·7 (2·9)	29·6 (4·5)	22·9 (4·7)	26·1 (2·6)	25·6 (4·6)
Homozygous HLA-DQ2·51	0	4 (50%)	0	1 (10%)	0	2 (29%)	7 (18%)

Data are mean (SD) or n (%). 1 "Homozygous HLA-DQ2·5" indicates no other HLA-DQA or HLA-DQB alleles detected apart from HLA-DQA1*05 & DQB1*02

Table 2: Adverse events graded at least moderate severity in participants experiencing ≥1 gastrointestinal adverse graded at least moderate severity

Treatment	Participant^	Gluten challenge in screening	Last dose number	Onset after last dose	Nausea	Abdominal Pain	Vomiting	Diarrhea	Other
3-dose study - weekly i.d. doses over 15 days									
Nexvax2 60 µg	S03-01-07	Yes	1	2.0-2.5h	+++	+++	+++	-	-
			2	3.5h	++	++	-	-	-
			3	3.5h	++	++	-	-	-
Nexvax2 90 µg	S03-02-13	Yes	1	3h	+++	++	+++	-	-
			2	2h	-	-	+	-	-
	S03-02-08	Yes	1	0.5-2.75h	++	++	++	-	
			2	2.5h	++	++	++	-	-
			3	2.5h	++	++	++	-	-
	S03-02-01	Yes	1	3.5h	++	-	++	-	-
			2	2.75h	-	+++	+++	+++	-
			3	2.25-3.25h	+++	+++	+++	+++	-
	S03-02-07	Yes	1	3-4h	++	++	++	++	+++1
	S03-02-10	Yes	1	3.25h	-	-	++	-	-
			2	2.4h	-	-	++	-	-
Nexvax2 150 µg	S03-03-03	Yes	1	4.3h	-	-	++	-	-
			2	3.2h	-	-	+++	-	-
	S03-03-02	Yes	1	4h	-	-	++	-	-
	S03-03-12	Yes	1	2.75-3.25h	++	-	++	-	-
			3	3h	-	-	++	-	-
	S03-03-09	Yes	1	3h	-	-	++	-	-
Placebo	S03-03-05	Yes	1	0	++	-	-	-	-
			2	24h	++	-	-	-	-
	S03-03-11	Yes	1	24h	-	-	-	-	+++2
16-dose study - twice weekly i.d. doses over 53 days									
Nexvax2 150 µg	S16-01-03	Yes	1	2.75h	-	-	++	-	-
	S16-01-04	Yes	1	2.4h	-	-	++	-	-
	S16-01-12	Yes	1	2.8-3.25h			+++		+++3
	S16-01-06	Yes	1	3h	-	-	++	-	-
Nexvax2 300 µg	S16-02-07	Yes	1	Same day	-	-	++	-	-
	S16-02-11	Yes	1	4.1h	-	-	++	-	-
	S16-02-12	Yes	1	3.1h	-	-	++	-	-
	S16-02-13	Yes	1	2.25h	-	++++	+	-	-
				48h	-	+++	++	-	-
Placebo	S16-02-04	Yes	14	24h			++		

^ Participant code (SXX-YY-ZZ) refers to the planned total doses in the study, the cohort number (YY), and order of randomization within the cohort (ZZ)

Adverse event grading +: mild; ++: moderate; +++: severe; ++++: serious (grade 4)

1 Dizziness; Adverse drug reaction, 2 Viral upper respiratory tract infection, 3 rigors

Table 3: Whole blood interferon-γ release assay (IGRA) with Nexvax2 peptides after post-treatment gluten challenge by post hoc analysis

Study	**3-dose study**				**16-dose study**		
Cohort/s	**1st, 2nd & 3rd**	**1st**	**2nd**	**3rd**	**1st & 2nd**	**1st**	**2nd**
Treatment	**Placebo**	**Nexvax 2 60 µg**	**Nexvax2 90 µg**	**Nexvax2 150 µg**	**Placebo**	**Nexvax2 150 µg**	**Nexvax2 300 µg**
Participants randomized	(n=11)	(n=9)	(n=9)	(n=8)	(n=7)	(n=8)	(n=10)
Participants commenced post-treatment OGC	11 (100%)	8 (89%)	8 (89%)	8 (100%)	7 (100%)	8 (100%)	2 (20%)
Participants completed post-treatment OGC	7 (78%)	6 (67%)	8 (89%)	7 (88%)	3 (43%)	8 (100%)	2 (20%)
Participants eligible for PD analysis*	9 (82%)	6 (67%)	8 (89%)	6 (75%)	5 (71%)	8 (100%)	2 (20%)
Responders of participants eligible for analysis	7 (78%)	4 (67%)	3 (38%)	0 (0%)	5 (100%)	2 (25%)	0 (0%)
P-value[#]	NA	1	0·1534	0·007	NA	0·021	0·0476

*Participants who did not finish all study doses, or post-treatment gluten challenge were not included in analysis. IGRA responders were Nexvax2-specific IGRA positive 6 or 8 days after commencing gluten challenge, and "non-responders" were Nexvax2-specific IGRA negative 6 or 8 days after commencing OGC when all 9 gluten cookies had been consumed (Algorithm outlined in Figures S3).

#P-value was estimated by Two-tailed Fisher's Exact Test comparing treatment with placebo

SUPPLEMENTARY APPENDIX

Epitope-specific immunotherapy targeting CD4+ T cells in celiac disease: evaluation in randomized, double-blind, placebo-controlled phase 1 studies

Gautam Goel, Tim King, A James Daveson, Jane M Andrews, Janakan Krishnarajah, Richard Krause, Gregor Brown, Ronald Fogel, Charles F Barish, Roger Epstein, Timothy P Kinney, Philip B Miner Jr, Jason A Tye-Din, Adam Girardin, Juha Taavela, Alina Popp, John Sidney, Markku Mäki, Kaela E Goldstein, Patrick H Griffin, Suyue Wang, John L Dzuris, Leslie J Williams, Alessandro Sette, Ramnik J Xavier, Ludvig M Sollid, Bana Jabri, Robert P Anderson.

TABLE OF CONTENTS

1. STUDY SITES

1.1 3-dose study

- IDT CMAX (Adelaide, South Australia, Australia
  - Principal Investigator – Assoc. Prof. Jane M Andrews
  - 9 participants randomized to treatment
- ClinSearch, LLC. (Chattanooga, Tennessee, USA)
  - Principal Investigator – Dr Richard Krause
  - 7 participants randomized to treatment
- Clinical Research Institute of Michigan (Chesterfield, Michigan, USA)
  - Principal Investigator – Dr Ronald Fogel
  - 5 participants randomized to treatment
- Wake Research Associates (Raleigh, North Carolina, USA)
  - Principal Investigator – Dr Charles H Barish
  - 4 participants randomized to treatment
- ActivMed Practices & Research (Newington, New Hampshire, USA)
  - Principal Investigator – Dr Roger Epstein
  - 4 participants randomized to treatment
- Q-Pharm Pty Ltd. (Herston, Queensland, Australia)
  - Principal Investigator – Dr A. James Daveson
  - 4 participants randomized to treatment
- Auckland Clinical Studies Ltd. (Auckland, New Zealand)
  - Principal Investigator – Dr Timothy King
  - 4 participants randomized to treatment
- Oklahoma Foundation for Digestive Research (Oklahoma City, Oklahoma, USA)
  - Principal Investigator – Dr Philip B Miner Jr
  - 2 participants randomized to treatment
- Prism Clinical Research (Waconia, Minnesota, USA)
  - Principal Investigator – Dr Timothy Kinney
  - 2 participants randomized to treatment
- Linear Clinical Research (Nedlands, Western Australia, Australia)
  - Principal Investigator – Dr Janakan Krishnarajah
  - 2 participants randomized to treatment

1.2 16-dose study

- Auckland Clinical Studies Ltd. (Auckland, New Zealand)
  - Principal Investigator – Dr Timothy King
  - 15 participants randomized to treatment
- Q-Pharm Pty Ltd. (Herston, Queensland, Australia)
  - Principal Investigator – Dr A. James Daveson
  - 12 participants randomized to treatment
- Linear Clinical Research (Nedlands, Western Australia, Australia)
  - Principal Investigator – Dr Janakan Krishnarajah
  - 6 participants randomized to treatment
- Nucleus Network (Melbourne Victoria, Australia)
  - Principal Investigator – Assoc. Prof. Gregor Brown5 participants randomized to treatment
- Christchurch Clinical Studies Trust Ltd. (Christchurch, New Zealand)
  - Principal Investigator – Dr Chris Wynne
  - 1 participant randomized to treatment

2. STUDY INDEPENDENT ETHICS COMMITTEES

2.1 3-dose study

- Liberty IRB Tracking #12.07.0012
- The University of Okalahoma Institutional Review Board for the Protection of Human Subjects IRB #1370
- Bellbury Human Research Ethics Committee, Application Number 2013-10-553

- Southern Health and Disability Ethics Committee 13/STH/168

2.2 16-dose study

- The Alfred Hospital Ethics Committee, Approval Number 118/12
- Bellbury Human Research Ethics Committee, Application Number 2012-04-735-AA
- Southern Health and Disability Ethics Committees, Ethics Ref. NTY/12/06/049/AM05

3. STUDY 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.

3.1 Inclusion Criteria

1. Patient has signed and understood the informed consent form (ICF) before initiation of any study specific procedures.
2. Patient is between 18 and 70 years old (inclusive).
3. Patient has confirmed "at risk" genotype (HLA-DQ2 and/or DQ8) and has a celiac disease diagnosis consistent with the criteria defined in the National Institutes of Health Consensus Statement 2004 [Department of Health and Human Services, 2004]:
    a. Diagnostic tests should be performed while the patient is on a gluten containing diet.
    b. A serologic antibody test should be positive.
    c. Patients with a positive celiac disease antibody test should undergo small bowel biopsy (those with biopsy-proven dermatitis herpetiformis can be excluded from small bowel biopsy).
        i. Multiple biopsies should be obtained (histologic changes may be focal) and include biopsies from the second portion of the duodenum or beyond.
    d. Some degree of villous atrophy should be observed.
4. Has HLA DQ2·5 genotype (both *DQA1*05* and *DQB1*02*, homozygous or heterozygous)

3.2 Exclusion Criteria

3.2.1 ***At Screening***

1. Patient possesses the genes encoding HLA DQ8 (either *DQA1*03* or *DQB1*0302*).
2. Patient has not been prescribed and/or has not followed a GFD for at least 12 months.
3. Patient has had known gluten exposure within two months prior to Screening.
4. Patient does not have a gluten specific T cell response (measured by IFN-γ release) following the Screening Period gluten challenge.
5. Patient is lactating or pregnant.
6. Patient is premenopausal, unless sterile, or using at least two acceptable birth control methods (Acceptable methods of birth control include tubal ligation, transdermal patch, intrauterine devices/systems, oral, implantable, or injectable contraceptives, sexual abstinence [if allowed by local authorities], double-barrier method, and vasectomized partner).
7. Patient is unable and/or unwilling to comply with study requirements.
8. Patient has had open abdominal surgery within the 12 months prior to Screening. (laparoscopic appendectomy and laparoscopic cholecystectomy within four months of Screening is allowed).
9. Patient has a positive test for human immunodeficiency virus or active hepatitis B or C disease at the time of Screening.
10. Patient has uncontrolled complications of celiac disease or unstable autoimmune disease which, in the opinion of the investigator, would impact the immune response or pose an increased risk to the patient.
11. Patient has uncontrolled peptic ulcer or gastroesophageal reflux disease or dyspepsia. The patient must be on a stable treatment regimen for their peptic ulcer or gastroesophageal reflux disease for two months prior to Screening.
12. Patient has insulin-dependent diabetes.
13. Patient has had treatment with systemic biological agents (e.g., adalimumab, etanercept, infliximab, certolizumab pegol) less than six months prior to Screening.
14. Patient has taken a nonsteroidal anti-inflammatory drug or aspirin within the past seven days prior to Screening. Daily low-dose aspirin therapy (up to 100 mg/day) is permitted.

15. Patient has taken oral corticosteroids within the previous six weeks prior to Screening. Inhaled steroids are acceptable.
16. Patient has taken systemic immunomodulatory agents (e.g., azathioprine, methotrexate) less than 30 days prior to Screening.
17. Patient has received an experimental therapy within 30 days prior to Screening.
18. Patient has been previously exposed to Nexvax2.
19. Patient has a history of clinically confirmed allergy and/or anaphylaxis to wheat, barley, or rye.
20. Patient has any of the following laboratory abnormalities at Screening:
    a. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), or alkaline phosphatase (ALP) >3 × the upper limit of normal (ULN)
    b. Hemoglobin <10 g/dL
    c. Platelet count <100× 109/L
    d. White blood cell count outside the normal range
    e. Thyroid-stimulating hormone outside the normal range
    f. Any other clinically significant abnormal laboratory values, as determined by the investigator
21. Patient is known to be pregnant, has a positive pregnancy test at Screening or Day 1 (Baseline), intends to become pregnant, or is nursing.
22. Patient has a history or presence of any medically significant condition considered by the investigator to have the potential to adversely affect participation in the study and/or interpretation of the study results.
23. Patient has a history of severe allergic reactions (e.g., swelling of the mouth and throat, difficulty breathing, hypotension, or shock) that require medical intervention.
24. Patient has donated blood ≤ 56 days prior to Screening.
25. Patient has a clinically relevant abnormality on electrocardiogram (ECG), as determined by the investigator.
26. Patient has inflammatory bowel disease (defined as ulcerative colitis or Crohn's disease).

3.2.2 ***Prior to randomization***

1. Patient has a positive gluten specific T cell response (measured by IFN-γ release assay) at the end of the Screening Period (**in ascending dose cohorts only)**
2. Patient has Screening small bowel mucosal biopsy histology consistent with a Marsh classification > Marsh 1 (**in biopsy cohorts only)**

4. DOSE ESCALATION CRITERIA

Dose-escalation was evaluated by the following criteria to determine if the study would proceed to the next cohort:

1. There are no more than two patients in a cohort with a local reaction to study drug injection that is > Grade 2.(1)
2. There are no more than two patients in a cohort with an abnormal vital sign (BP, T, HR) that is > Grade 2.(1)
3. There are no more than two patients in a cohort with vomiting, diarrhea, headache fatigue or myalgia that is > Grade 2.(1)
4. There are no more than two patients in a cohort with any symptom recorded in the daily GI symptom diary that reached the following threshold: rated as "severe" or "very severe" for more than 2 days per week of two consecutive weeks and represents at least one level of severity increase for the worst week recorded during the Screening Period for that symptom.
5. There are no more than two patients in a cohort with any symptom recorded in the weekly GI symptom dairy that reached the following threshold: rated as "severe" or "very severe" for more than 2 days per week of two consecutive weeks and represents at least one level of severity increase for the worst week recorded during the Screening Period for that symptom.
6. There are no more than two patients in a cohort with elevations in AST or ALT Grade 2 (≥ 5.1 x ULN) or greater that do not return to normal or near normal (Grade 1) by Day 15. AST and ALT of ≥ Grade 2 triggered a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing

schedule proceeded if the confirmatory lab values indicated a toxicity of < Grade 2. Grading was established according to Guidance for Industry.(1)

7. There are no more than two patients in a cohort with elevations in ALP Grade 2 (≥ 3.1 x ULN) or greater that do not return to normal or near normal (Grade 1) by Study Day 15. ALP of ≥ Grade 2 will trigger a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing schedule may proceed if the confirmatory lab values indicate a toxicity of < Grade 2. Grading will be established according to Guidance for Industry.(1)
8. There are no more than two patients in a cohort with abnormal clinical chemistry laboratory tests, exclusive of alkaline phosphatase (ALP), AST, or ALT, that was ≥ Grade 2, according to Guidance for Industry (Appendix B of protocol). Abnormal clinical chemistry laboratory tests of ≥ Grade 2 triggered a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing schedule proceeded if the confirmatory lab values indicated a toxicity of < Grade 2.
9. There are no more than two patients in a cohort with abnormal hematology laboratory tests (i.e., WBC, Hgb, lymphocyte, neutrophils), that are ≥ Grade 2, according to Guidance for Industry.(1) Abnormal hematology laboratory tests of ≥ Grade 2 triggered a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing schedule proceeded if the confirmatory lab values indicated a toxicity of < Grade 2.
10. There are no more than two patients in a cohort with abnormal urinalysis laboratory tests (i.e., protein, glucose, microscopic blood), that are ≥ Grade 2, according to Guidance for Industry.(1) Abnormal urinalysis laboratory tests of ≥ Grade 2 triggered a repeat test for confirmation of the value within 48 hours. Escalation to the next dosing schedule proceeded if the confirmatory lab values indicate a toxicity of < Grade 2.
11. There are no patients in a cohort with cardiovascular, respiratory, neurological, musculoskeletal, skin, infectious, otolaryngologic, or system toxicities that required ER visit or hospitalization.

5. METHODS

5.1 Development of Nexvax2

To develop Nexvax2, the specificities of polyclonal gluten-specific T cells circulating in blood of HLA-DQ2·5+ celiac patients after feeding them wheat, barley or rye were assessed in quantitative overnight IFN-γ ELISpot assays(2). The peptide composition of Nexvax2 was determined after screening 16,838 unique 12–amino acid oligopeptides in 313 GenBank entries for gliadins, LMW glutenins, and HMW glutenins from Triticum aestivum after wheat challenge, hordeins from Hordeum vulgare after barley challenge, and secalins from Secale cerale after rye challenge. T cell clones raised to the three peptides selected for inclusion in Nexvax2 responded to 61 of the 96 immunoreactive peptides identified from screening a peptide library encompassing 16,838 unique 12–amino acid sequences. T cell responses to these 3 peptides were additive when mixed together and assessed in IFN-γ ELISpot assays using peripheral blood mononuclear cells (PBMC) from CeD donors.

The peptides in Nexvax2, NPL001, NPL002 and NPL003 correspond to partially deamidated germline-encoded sequences in certain wheat α-gliadins, wheat ω-gliadins/barley C-hordeins, or barley B-hordeins with synthetically modified N- and C-termini. In vitro assays performed by the La Jolla Institute of Allergy and Immunology (La Jolla, California, USA) assessed the binding of NPL001, NPL002, and NPL003 to isolated MHC Class II molecules according to established methods(3). Each peptide binds selectively with intermediate affinity to HLA-DQ2·5 (Table S1). The equimolar mixture of peptides in Nexvax2 stimulates concentration-dependent secretion of IFN-γ, and IL-10 by CD4+ T cell clones specific for epitopes in Nexvax2 (Figure S1A-E),(4) attenuated by co-incubation with anti-HLA-DQ, but not anti-HLA-DR (Figure S1F-J).

Conduct of these phase 1 studies were supported by preclinical studies of Nexvax2 investigating pharmacodynamics in HLA-DR3-DQ2·5 transgenic mice, including a related strain that was additionally T-cell receptor transgenic with CD4+ T cells specific for the DQ2·5-glia-α2 epitope present in Nexvax2,(5) toxicology and PK studies in rodents, clinical medicinal chemistry studies, the prior first-in-human study of Nexvax2, and clinical studies of peptide-based therapeutic vaccines.

5.2 Investigational drug product

CS Bio (Menlo Park, California, USA) manufactured NPL001, NPL002, and NPL003. Microtest (Agawam, Massachusetts, USA) formulated and filled vials with a sterile equimolar solution at total peptide concentration 9 mg/mL in sterile USP 0.9% sodium chloride. Placebo and diluent for Nexvax2 in vials was USP 0.9% sodium chloride. Placebo or Nexvax2 150 μg (50 μg of each peptide), or 300 μg in 0.1 mL were administered by 1 mL Luer-Lok™ plastic syringe fitted with a Micro Injection Needle (Becton-Dickinson). Grand River Aseptic Manufacturing (Grand Rapids, Michigan, USA) formulated and filled Soluvia™ syringes (Becton-Dickinson, Franklin Lakes, New Jersey, USA) with Nexvax2 (0.6 mg/mL, 0.9 mg/mL, or 1.5 mg/mL) or placebo.

5.3 Lab procedures for clinical trials

5.3.1 *Safety laboratory pathology assessments*

Laboratory assessments included routine haematology, blood chemistry, and urinalysis performed by Dorevitch Pathology for sites in Australia in New Zealand, and by LabConnect (Johnson City, Tennessee, USA) for sites in the United States.

5.3.2 *Whole blood interferon-γ release assay (IGRA)*

IFN-γ levels in plasma separated from whole blood incubations for IGRA collected during the screening periods of each study were measured by ELISA performed either at ImmusanT, Inc. for samples from sites in the USA, or at the Walter and Eliza Hall Institute (Parkville, Vicotria, Australia) for sites in Australia or New Zealand. After each study was completed, thawed plasma from all whole blood IGRA incubations were re-assessed by IFN-γ ELISA at ImmusanT, which were regarded as the final, reported IGRA data. Briefly, 1 mL of blood was collected into each of three Nil Control Tubes (QuantiFERON®-TB Gold In-Tube, QIAGEN, Hilden, Germany) that had 0.1 mL phosphate buffer saline (PBS) alone, Nexvax2 peptides (each 50 μg/mL), or positive control CEF peptide pool with epitopes derived from cytomegalovirus, Epstein-Barr virus, and influenza (0.1 μg/mL; Mabtech AB, Nacka Strand, Sweden) added, and a QuantiFERON Mitogen Tube with 0.1 mL PBS added. Tubes were incubated at 37°C for 24 h before centrifugation, and IFN-γ in the supernatant was measured by ELISA (Mabtech). To evaluate the magnitude of responses, a stimulation index (SI) was calculated for the average IFN-γ concentrations in the CEF and Nexvax2-peptide incubations divided by the concentration determined for the response to PBS alone. A "positive" response was defined as SI>1.25 and net IFN-γ concentration above PBS control >7.2pg/mL.(6, 7)

5.3.3 *Plasma concentrations of cytokines and chemokines*

Blood was collected into K2 EDTA Vacutainer® tubes, which were immediately placed on wet ice, and then centrifuged at 1100-1300 RCF for 10 minutes within 30 minutes of collection. Plasma was aliquotted and frozen. Cytokines and chemokines were measured in plasma using a 38plex magnetic bead-based assay according the manufacturer's protocol (EMD Millipore Corp., Billerica, MA; Luminex Corporation, Austin, Texas, USA) at ImmusanT, Inc.

5.3.4 *Plasma concentrations of complement components*

Complement levels were measured in plasma collected for cytokine/chemokine assessment by magnetic bead-based assay according the manufacturer's protocol (Milliplex® MAP Human Complement Magnetic Bead Panel 1 and 2) at ImmusanT, Inc.

5.3.5 *Immune cell profiling*

PBMC were prepared at trial sites according to manufacturer's instructions using Ficoll-Paque™ PLUS (Sigma-Aldrich) in SepMate™-50 tubes (STEMCELL Technologies Inc.; Vancouver, BC, Canada), and cryopreserved using CryoStor™ CS10 (STEMCELL Technologies Inc.). Flow cytometry was performed at Duke Center for AIDS Research Flow Cytometry Core Facility (Durham, North Carolina, USA) using pre-mixed labeled antibodies specific for CD3, CD4, CD8, CD45, CD16, CD56, and CD19 according to established protocols(8-11).

5.3.6 ***Pharmacokinetics***

Pharmacokinetics were assessed on the first and last days of dosing. Blood was collected 30 minutes before, and 10, 20, 30, and 45 minutes, and 1, 1.5, 2, 4, and 6 hours after dosing. Blood was collected into K2 EDTA Vacutainer® tubes (Becton-Dickinson), which were centrifuged at 1100-1300 RCF for 10 minutes within 10min of collection. Plasma samples were spiked with isotopically labeled Nexvax2 peptides (50 ng/mL; Pepscan, Lelystad, The Netherlands), extracted using C18 Sep-Pak SPE cartridges, and analyzed by high-performance liquid chromatography with tandem mass spectrometric developed and performed by Blue Stream Laboratories (Woburn, Massachusetts, USA).

5.3.7 ***Celiac disease serology***

Assays for CeD serology in sera collected for anti-Nexvax2 antibodies was performed by Healthscope Pathology (Clayton, Victoria, Australia) using QUANTA Lite® R h-tTG IgA, Gliadin IgA II [DGP], Gliadin IgG II [DGP] kits (INOVA Diagnostics, San Diego, California, USA).

5.3.8 ***Duodenal histology***

The central pathologist (Dorevitch Pathology; Heidelberg VIC, Australia) evaluated biopsies in the screening period to determine eligibility. After the end of each study, the central pathologist masked to the order that biopsies were collected, re-evaluated all biopsies to make a final assessment of modified Marsh type. Histology slides were shipped to the University of Tampere, where villous height to crypt depth (VH:CrD) ratio and intra-epithelial lymphocytes (IEL) density per 100 epithelial cells were measured in well oriented sections.(12)

5.4 Major histocompatibility class II peptide binding

In vitro assays performed by the La Jolla Institute of Allergy and Immunology (La Jolla, California, USA) assessed the binding of NPL001, NPL002, and NPL003 to isolated MHC Class II molecules according to established methods(3).

6. STATISTICAL METHODS FOR POST HOC ANALYSES

6.1 Primary endpoints

Two-tailed Fisher's Exact Test was used to compare (1) number of participants who experienced treatment emergent adverse events in placebo and active arms, and (2) number of Nexvax2-treated participants who experienced severe adverse events stratified by HLA-DQ2.5 homozygosity status.

6.2 Secondary endpoints

Wilcoxon Rank Sum test was used to compare individual and summed daily symptom scores for each participant after dosing relative to pre-dose baseline scores. Wilcoxon Signed-Rank test was used to compare (1) weekly GSRS scores between a treatment week and the baseline week (Table S7), (2) daily symptoms scores between gluten challenge day and placebo challenge day during screening period (Table S8), and (3) change in percentage lymphocytes from day 1 of treatment on other days (Table S9).

6.3 Exploratory endpoints

Villous height to crypt depth (VH:CrD) ratio and intra-epithelial lymphocytes (IEL) density pre- and post-treatment were analyzed by Wilcoxon Signed Rank test (Table S11).

6.4 Pharmacodynamic endpoints

To address the confounding effects of reduced gluten exposure in the post-treatment OGC, an algorithm was developed post hoc to define the populations for post-treatment symptom and pharmacodynamic analysis (Figure S3). Two-tailed Fisher's Exact Test was used to compare (1) number of Nexvax2 and placebo treated participants who finished the post-treatment OGC, and (2) number of Nexvax2 and placebo treated participants who had negative IGRA at day six or eight after commencing post-treatment OGC (Table 3).

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Table S1: Nexvax2 composition and binding to HLA-DQ molecules associated with celiac disease

Nexvax2 Peptide	Amino acids	HLA-DQ2·5-restricted T-cell epitopes	9 amino acid epitope sequences	Major Histocompatibility Class II binding affinity (IC50 nM)		
				HLA-DQ2·5	HLA-DQ2·2	HLA-DQ8
NPL001	16	DQ2·5-glia-α1a	PFPQPELPY	Intermediate	Low	Negligible
		DQ2·5-glia-α2	PQPELPYPQ	(109 nM)	(1778 nM)	(>5000 nM)
NPL002	15	DQ2·5-glia-ω1	PFPQPEQPF	Intermediate	Negligible	Negligible
		DQ2·5-glia-ω2	PQPEQPFPW	(87 nM)	(>5000 nM)	(>5000 nM)
NPL003	16	DQ2·5-hor-3	PIPEQPQPY	Intermediate	Low	Negligible
		var DQ2·5-glia-γ5	EQPIPEQPQ	(231 nM)	(1405 nM)	(>5000 nM)

Table S2. Treatment-emergent adverse events that occurred during the treatment period in the 3-dose study

Treatment-Emergent Adverse Events (TEAE)	Treatment-emergent adverse events in 3-dose study						
	1st, 2nd & 3rd	1st	2nd	3rd	4th (biopsy)	4th (biopsy)	All Participants Dosed
	Placebo	Nexvax2	Nexvax2	Nexvax2	Placebo	Nexvax2	
		60µg	90µg	150µg		150µg	
	(N=11)	(N=9)	(N=9)	(N=8)	(N=3)	(N=3)	(N=43)
	Number (%) of participants						
Number of participants with TEAEs	6 (55%)	5 (56%)	7 (78%)	5 (63%)	3 (100%)	1 (33%)	27 (63%)
Number of participants with study drug-related TEAEs	4 (36%)	5 (56%)	6 (67%)	3 (38%)	1 (33%)	1 (33%)	20 (47%)
Number of participants with moderate or severe TEAEs	3 (27%)	3 (33%)	5 (56%)	4 (50%)	1 (33%)	0	16 (37%)
Number of participants with study drug-related, moderate or severe TEAEs	2 (18%)	3 (33%)	5 (56%)	3 (38%)	1 (33%)	0	14 (33%)
Number of participants withdrawn due to TEAEs	0	0	1 (11%)	0	0	0	1 (2%)
Number of participants with a treatment-emergent serious adverse events	1 (9%)	0	0	0	0	0	0
	Number of events						
Number of TEAEs	15	25	65	16	7	1	129
Number of study drug-related TEAEs	10	22	60	11	1	1	105
Number of moderate or severe TEAEs	4	18	47	9	1	0	79
Number of study drug-related, moderate or severe TEAEs	3	15	46	8	1	0	73
Number of TEAEs leading to withdrawal	0	0	1	0	0	0	1
Number of treatment-emergent serious adverse events	4	0	0	0	0	0	0

Table S3. Treatment-emergent adverse events that occurred during the treatment period in the 16-dose study

Treatment-Emergent Adverse Events (TEAE)	Treatment-emergent adverse events in 16-dose study					
	1st & 2nd	1st	2nd	3rd (biopsy)	3rd (biopsy)	All Participants Dosed
	Placebo	Nexvax2	Nexvax2	Placebo	Nexvax2	
		150 µg	300 µg		150 µg	
	(N=7)	(N=8)	(N=10)	(N=7)	(N=7)	(N=39)
	Number (%) of participants					
Number of participants with TEAEs	5 (71%)	6 (75%)	10 (100%)	6 (86%)	5 (71%)	32 (82%)
Number of participants with study drug-related TEAEs	3 (43%)	5 (63%)	9 (90%)	3 (43%)	4 (57%)	24 (62%)
Number of participants with moderate or severe TEAEs	1 (14%)	5 (63%)	8 (80%)	0	2 (29%)	16 (41%)
Number of participants with study drug-related, moderate or severe TEAEs	0	5 (63%)	8 (80%)	0	1 (14%)	14 (36%)
Number of participants withdrawn due to TEAEs	0	0	3 (30%)	0	0	3 (8%)
Number of participants with a treatment-emergent SAE	0	0	1 (10%)	0	0	1 (3%)
	Number of events					
Number of TEAEs	13	21	26	24	18	102
Number of study drug-related TEAEs	5	16	16	5	7	49
Number of moderate or severe TEAEs	1	8	12	0	3	24
Number of study drug-related, moderate or severe TEAEs	0	7	8	0	2	17
Number of TEAEs leading to withdrawal	0	0	3	0	0	3
Number of treatment-emergent serious adverse events	0	0	1	0	0	1

Table S4. Treatment emergent adverse events that occurred during the treatment period in at least 5% of particpants in the 3-dose study.

System Organ Class, Preferred Term	Treatment-emergent adverse events (TEAE)						
	1st, 2nd & 3rd Placebo	1st Nexvax2 60µg	2nd Nexvax2 90µg	3rd Nexvax2 150µg	4th (biopsy) Placebo	4th (biopsy) Nexvax2 150µg	All Particpants Dosed
	(N=11)	(N=9)	(N=9)	(N=8)	(N=3)	(N=3)	(N=43)
Number (%) of particpants with at least 1 TEAE	6 (55%)	5 (56%)	7 (78%)	5 (63%)	3 (100%)	1 (33%)	27 (63%)
[Number of TEAEs]	[15]	[25]	[65]	[16]	[7]	[1]	[129]
Gastrointestinal Disorders	2 (18%)	2 (22%)	5 (56%)	4 (50%)	1 (33%)	0	14 (33%)
	[4]	[9]	[36]	[8]	[3]		[60]
Vomiting	0	2 (22%)	5 (56%)	4 (50%)	0	0	11 (26%)
		[2]	[12]	[6]			[20]
Nausea	1 (9%)	1 (11%)	4 (44%)	2 (25%)	1 (33%)	0	9 (21%)
	[2]	[3]	[9]	[2]	[1]		[17]
Abdominal pain	0	1 (11%)	3 (33%)	0	1 (33%)	0	5 (12%)
		[3]	[5]		[1]		[9]
Abdominal tenderness	0	0	2 (22%)	0	0	0	2 (5%)
			[2]				[2]
Diarrhoea	0	0	2 (22%)	0	0	0	2 (5%)
			[4]				[4]
General Disorders and Administration Site Conditions	2 (18%)	4 (44%)	3 (33%)	1 (13%)	0	0	10 (23%)
	[3]	[10]	[7]	[1]			[21]
Injection site pain	0	3 (33%)	1 (11%)	0	0	0	4 (9%)
	[0]	[8]	[2]				[10]
Fatigue	1 (9%)	0	2 (22%)	0	0	0	3 (7%)
	[1]		[2]				[3]
Infections and Infestations	3 (27%)	1 (11%)	1 (11%)	1 (13%)	2 (67%)	0	8 (19%)
	[3]	[1]	[1]	[1]	[2]		[8]
Urinary tract infection	0	0	1 (11%)	0	1 (33%)	0	2 (5%)
			[1]		[1]		[2]
Viral upper respiratory tract infection	1 (9%)	0	0	1 (13%)	0	0	2 (5%)
	[1]			[1]			[2]
Nervous System Disorders	1 (9%)	1 (11%)	4 (44%)	1 (13%)	0	0	7 (16%)
	[3]	[1]	[8]	[1]			[13]
Headache	0	0	4 (44%)	0	0	0	4 (9%)
			[7]				[7]
Dizziness	0	0	1 (11%)	1 (13%)	0	0	2 (5%)
			[1]	[1]			[2]
Musculoskeletal and Connective Tissue Disorders	0	1 (11%)	2 (22%)	1 (13%)	0	0	4 (9%)
		[1]	[2]	[2]			[5]
Myalgia	0	1 (11%)	1 (11%)	0	0	0	2 (5%)
		[1]	[1]				[2]

Table S5. Treatment emergent adverse events that occurred during the treatment period in at least 5% of particpants in the 16-dose study

System Organ Class, Preferred Term	Treatment-emergent adverse events (TEAE)					
	1st & 2nd	1st	2nd	3rd (biopsy)	3rd (biopsy)	All Particpants Dosed
	Placebo	Nexvax2	Nexvax2	Placebo	Nexvax2	
		150 µg	300 µg		150 µg	
	(N=7)	(N=8)	(N=10)	(N=7)	(N=7)	(N=39)
Number (%) of particpants with at least 1 TEAE	5 (71%)	6 (75%)	10 (100%)	6 (86%)	5 (71%)	32 (82%)
[Number of TEAEs]	[13]	[21]	[26]	[24]	[18]	[102]
Nervous System Disorders	5 (71%)	3 (38%)	5 (50%)	4 (57%)	3 (43%)	20 (51%)
	[7]	[10]	[9]	[6]	[4]	[36]
Headache	3 (43%)	3 (38%)	5 (50%)	3 (43%)	2 (29%)	16 (41%)
	[3]	[9]	[8]	[4]	[2]	[26]
Dizziness	2 (29%)	0	0	1 (14%)	0	3 (8%)
	[4]			[1]		[5]
Migraine	0	1 (13%)	1 (10%)	0	0	2 (5%)
		[1]	[1]			[2]
Lethargy	0	0	0	1 (14%)	1 (14%)	2 (5%)
				[1]	[1]	[2]
Gastrointestinal Disorders	1 (14%)	5 (63%)	6 (60%)	3 (43%)	2 (29%)	17 (44%)
	[1]	[5]	[10]	[5]	[3]	[24]
Vomiting	0	5 (63%)	4 (40%)	1 (14%)	0	10 (26%)
		[5]	[5]	[1]		[11]
Abdominal pain	0	0	1 (10%)	1 (14%)	0	2 (5%)
			[2]	[1]		[3]
Diarrhea	0	0	0	1 (14%)	1 (14%)	2 (5%)
				[1]	[1]	[2]
Dry mouth	0	0	0	1 (14%)	1 (14%)	2 (5%)
				[1]	[1]	[2]
Gastrointestinal disorder	0	0	2 (20%)	0	0	2 (5%)
			[2]			[2]
General Disorders and Administration Site Conditions	1 (14%)	3 (38%)	1 (10%)	1 (14%)	3 (43%)	9 (23%)
	[1]	[3]	[1]	[1]	[4]	[10]
Vessel puncture site haematoma	1 (14%)	0	0	0	1 (14%)	2 (5%)
	[1]				[2]	[3]
Fatigue	0	1 (13%)	0	0	1 (14%)	2 (5%)
		[1]			[1]	[2]
Infections and Infestations	1 (14%)	1 (13%)	1 (10%)	4 (57%)	0	7 (18%)
	[1]	[1]	[1]	[5]		[8]
Upper respiratory tract infection	0	0	0	3 (43%)	0	3 (8%)
				[3]		[3]
Nasopharyngitis	0	0	1 (10%)	1 (14%)	0	2 (5%)
			[1]	[1]		[2]
Pharyngitis	0	1 (13%)	0	1(14%)	0	2 (5%)
		[1]		[1]		[2]
Musculoskeletal and Connective Tissue Disorders	0	0	2 (20%)	1 (14%)	1 (14%)	4 (10%)
			[2]	[2]	[1]	[5]

Back pain	0	0	2 (20%) [2]	1 (14%) [1]	0	3 (8%) [3]
Respiratory, Thoracic and Mediastinal Disorders	0	0	2 (20%) [2]	2 (29%) [4]	0	4 (10%) [6]
Oropharyngeal pain	0	0	1 (10%) [1]	1 (14%) [1]	0	2 (5%) [2]

Table S6. Moderate or severe adverse events, and any occurrence of vomiting in the treatment period

Participant	Treatment	Age	Sex	HLA-DQ2.5 Homozygote	Gluten challenge in screening	Total doses received	Last dose	Onset after last dose	Adverse event	Severity
3-dose study										
S03-01-01	Nexvax2 60 µg	49	F	No	Yes	3	1	5h 20m	Vomiting x1	Mild
S03-01-09	Nexvax2 60 µg	63	F	Homozygote	Yes	3	1	1 day	Worsening of Diabetes Mellitus type 2; Worsening of lower limb edema	Moderate
								3 days	Rash on right forearm "Redness and itching about 5 inch diameter"	Moderate
							2	1 day	Muscle aches both lower limbs	Moderate
							3	0	Worsening of seasonal allergy	Moderate
S03-01-07	Nexvax2 60 µg	36	F	Homozygote	Yes	3	1	0	Burning at Injection Site; Soreness in left arm (near injection site)	Moderate
								2h	Nausea; Abdominal Pain	Severe
								2h 30m	Vomiting	Severe
							2	0	Burning at injection site	Moderate
								3h 30m	Nausea; Abdominal Pain	Moderate
							3	0:00	Constipation	Moderate
								3h 30m	Nausea; Abdominal Pain	Moderate
S03-01-05	Placebo	64	M	Homozygote	Yes	3	1	1 day	Worsening Rash on Right Back (Perivascular Dermatitis)	Moderate
S03-02-13	Nexvax2 90 µg	42	F	Homozygote	Yes	3	1	3h	Nausea; Vomiting	Severe
								3h	Abdominal pain	Moderate
							2	2h	Vomiting	Mild
S03-02-08	Nexvax2 90 µg	29	F	No	Yes	3	1	30m	Flushing; Aggravated Headache; Nausea	Moderate
								2h 45m	Vomiting; Right sided abdominal pain	Moderate
							2	30m	Flushing; Aggravated Headache	Moderate
								2h 30m	Nausea; Vomiting; Right sided abdominal pain	Moderate
							3	30m	Flushing; Aggravated Headache	Moderate
								2h 30m	Nausea; Vomiting; Right sided abdominal pain	Moderate
S03-02-01	Nexvax2 90 µg	58	F	No	Yes	3	1	0	Burning at Injection Site	Moderate
								3h 30m	Nausea; Vomiting; Generalized Weakness	Moderate
							2	2h 45m	Vomiting; Diarrhea; Abdominal Spasms	Severe
								1 day	Muscle aches bilateral legs; Soft Tissue Swelling Left Foot	Moderate
							3	2h 15m	Nausea	Severe
								3h 15m	Vomiting; Diarrhea; Abdominal Pain	Severe
S03-02-07 ❋	Nexvax2 90 µg	62	F	Homozygote	Yes	1	1	2h 30m	Headache	Moderate
								3h	Nausea; Diaphoresis	Moderate
								3h	Dizziness; Adverse drug reaction	Severe
								3h	Lip dysesthesia; Dysphagia (lump in throat upon swallowing)	Moderate
								3h 30m	Abdominal pain & tenderness	Moderate
								4h	Vomiting; Diarrhea	Moderate
								5h 30m	Diffuse arthralgia	Moderate
								7h 30m	Sinus congestion	Severe

								7h 30m	Cough	Moderate
								5 days	Sinus congestion	Moderate
S03-02-10	Nexvax2 90 µg	43	M	Homozygote	Yes	3	1	3h 15m	Vomiting	Moderate
							2	2h 25m	Vomiting	Moderate
							3	2h 20m	Vomiting	Mild
S03-03-03	Nexvax2 150 µg	48	M	No	Yes	3	1	4h 20m	Vomiting	Moderate
							2	3h 10m	Vomiting	Severe
								3h 30m	Clammy skin	Severe
								5h	Shivering	Severe
S03-03-02	Nexvax2 150 µg	18	F	No	Yes	3	1	4h	Vomiting	Moderate
S03-03-12	Nexvax2 150 µg	52	F	No	Yes	3	1	2h 45m	Nausea	Moderate
								3h 15m	Vomiting	Moderate
							3	3h	Vomiting	Moderate
S03-03-09	Nexvax2 150 µg	27	F	No	Yes	3	1	3h	Vomiting	Moderate
S03-03-05	Placebo	49	F	No	Yes	3	1	0	Nausea	Moderate
							2	1 day	Nausea	Moderate
S03-03-11	Placebo	31	F	No	Yes	3	1	1 day	Viral upper respiratory tract infection	Severe
S03-04-06	Placebo	53	F	No	No	3	2	6 days	Insomnia	Moderate
16-dose study										
S16-01-02	Nexvax2 150 µg	64	F	No	Yes	16	3	1 day	Fatigue	Moderate
S16-01-03	Nexvax2 150 µg	51	F	Homozygote	Yes	16	1	2h 45m	Vomiting	Moderate
							3	1 day	Headache	Moderate
S16-01-04	Nexvax2 150 µg	54	F	Homozygote	Yes	16	1	2h 25m	Vomiting	Moderate
S16-01-08	Nexvax2 150 µg	60	F	No	Yes	16	1	3 days	Vomiting	Mild
S16-01-12	Nexvax2 150 µg	40	M	Homozygote	Yes		1	2h 50m	Vomiting	Severe
								4h 15m	Rigors	Severe
S16-01-06	Nexvax2 150 µg	66	F	UNK	Yes	16	1	3h	Vomiting	Moderate
							4	2 days	Headache	Moderate
S16-02-05	Nexvax2 300 µg	64	F	No	Yes	16	9	1 day	Headache	Moderate
S16-02-07 ¶	Nexvax2 300 µg	48	F	No	Yes	1	1	Same day	Vomiting	Moderate
								7h 20m	Headache	Moderate
S16-02-01	Nexvax2 300 µg	28	M	UNK	Yes	16	3	2 days	Headache	Moderate
S16-02-02 □	Nexvax2 300 µg	54	M	No	Yes	1	1	3h 15m	Gastrointestinal reaction to study drug	Moderate
S16-02-03 □	Nexvax2 300 µg	58	F	No	Yes	2	2	3h 50m	Gastrointestinal reaction to study drug	Moderate
S16-02-11	Nexvax2 300 µg	50	F	No	Yes	4	1	4h 5m	Vomiting	Moderate
S16-02-12	Nexvax2 300 µg	55	F	No	Yes	4	1	3h 5m	Vomiting	Moderate
S16-02-13 §	Nexvax2 300 µg	45	M	Homozygote	Yes	1	1	2h 15m	Abdominal Pain	Severe
								2h 15m	Vomiting	Mild
								2 days	Abdominal Pain	Severe
								2 days	Vomiting	Moderate
S16-02-04	Placebo	35	M	No	Yes	15	14	1 day	Vomiting	Moderate
S16-03-02	Nexvax2 150 µg	37	M	Homozygote	No	16	4	7h 30m	Headache	Moderate
S16-03-11	Nexvax2 150 µg	44	F	No	No	16	12	3h	Fatigue	Moderate
								4h 45m	Bilateral thigh muscle pain	Moderate
S16-03-08	Placebo	21	F	No	No	16	14	7h 25m	Vomiting	Mild

 Participant code (SXX-YY-ZZ) refers to the planned total doses in the study, the cohort number (YY), and order of randomization within the cohort (ZZ)

❄ S03-02-07 experienced diarrhea at approximately 3 hours following dosing, felt very faint, severely nauseated, and became very cold and pale. Study treatment was discontinued.

¶ S16-02-07 was discontinued from study treatment as GI symptoms were poorly tolerated.

. S16-02-02 and S16-02-03 had "Gastrointestinal reaction to study drug" including vomiting (MedDRA term of GI disorder) resulting in S16-02-02 discontinuing after 1st dose andand S16-02-03 after 2nd dose

§ S16-02-13 was discontinued after first dose.

Table S7. Weekly GSRS scores

	Weekly gastrointestinal symptom rating scale score					
Study	**3-dose study**					
Cohort/s	**1st, 2nd & 3rd**	**1st**	**2nd**	**3rd**	**4th (biopsy)**	**4th (biopsy)**
Treatment	**Placebo**	**Nexvax2**	**Nexvax2**	**Nexvax2**	**Placebo**	**Nexvax2**
Dose		**60µg**	**90µg**	**150µg**		**150µg**
N	**11**	**9**	**90µg**	**8**	**3**	**3**
Pre-treatment						
Week of gluten challenge	1·91 (1·17)	2·18 (0·56)	2·04 (1·16)	1·82 (0·86)	1·20 (0·24)	1·04 (0·04)
Week of placebo challenge	1·60 (0·53)	1·88 (0·67)	1·98 (0·91)	1·22 (0·41)	1·22 (0·10)	1·13 (0·12)
Last week of screening (baseline)	1·33 (0·41)	1·72 (0·68)	1·59 (0·75)	1·19 (0·23)	1·18 (0·10)	1·04 (0·08)
Treatment						
Treatment Week 1	1·35 (0·41)	1·77 (0·83)	2·36 (1·47) (p = 0·0313)	1·33 (0·35)	1·20 (0·00)	1·11 (0·10)
Treatment Week 2	1·28 (0·35)	1·93 (0·94)	1·75 (0·63)	1·37 (0·37)	1·22 (0·20)	1·03 (0·05)
Study	**16-dose study**					
Cohort/s	**1**	**1**	**2**	**2**	**7**	**7**
Treatment	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**
Dose		**150 µg**		**300 µg**		**150 µg**
N	**4**	**8**	**3**	**10**	**7**	**7**
Pre-treatment						
Week of gluten challenge	2·03 (0·80)	1·65 (0·78)	1·62 (0·56)	1·66 (0·55)	1·30 (0·32)	1·36 (0·30)
Week of placebo challenge	1·62 (0·40)	1·26 (0·31)	1·60 (0·07)	1·45 (0·34)	1·29 (0·22)	1·24 (0·21)
Last week of screening (baseline)	1·58 (0·59)	1·24 (0·38)	1·11 (0·14)	1·29 (0·32)	1·25 (0·25)	1·27 (0·30)
Treatment						
Treatment Week 1	1·75 (0·55)	1·90 (0·99) (p = 0·0313)	1·16 (0·27)	1·77 (0·52) (p = 0·0078)	1·36 (0·26)	1·59 (0·54) (p = 0·0313)
Treatment Week 7	1·25 (0·30)	1·32 (0·41)	1·60 (0·00)	1·17 (0·05)	1·41 (0·35)	1·32 (0·26)

Data are mean (SD). P-value was estimated by Wilcoxon Signed Rank test between a treatment week and the baseline week. Significant values ($p <= 0·05$) are highlighted in red.

Table S8. Daily symptoms diary scores during pre-treatment screening

Cohort	Day	Pain or Discomfort		Hunger Pain		Nausea		Rumbling		Bloating		Diarrhea	
		Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week	Gluten Challenge Week	Placebo Challenge Week
All screened with OGC (N = 95)	Challenge Day 1	1·84 (1·18) (p = 0·0047)	1·46 (0·74)	1·41 (0·81)	1·42 (0·87)	1·91 (1·68) (p = 0·0011)	1·32 (0·82)	1·74 (0·99) (p = 0·0157)	1·46 (0·80)	2·03 (1·19) (p = 0·0491)	1·76 (1·10)	1·56 (1·21) (p = 0·0224)	1·26 (0·72)
	Challenge Day 2	1·99 (1·26) (p = 0·0326)	1·64 (0·92)	1·36 (0·71)	1·27 (0·68)	1·65 (1·15) (p = 0·0279)	1·34 (0·74)	1·76 (1·04)	1·58 (0·89)	2·44 (1·50) (p = 0·0046)	1·91 (1·19)	1·57 (1·11)	1·32 (0·76)
	Challenge Day 3	1·95 (1·34) (p = 0·0370)	1·62 (1·05)	1·41 (0·88)	1·26 (0·61)	1·81 (1·34) (p = 0·0014)	1·33 (0·75)	1·73 (1·05)	1·52 (0·90)	2·47 (1·52) (p = 0·0005)	1·82 (1·18)	1·55 (1·04)	1·34 (0·83)
	Day 4	1·69 (1·12)	1·57 (1·14)	1·41 (0·81) (p = 0·0192)	1·24 (0·61)	1·40 (0·89)	1·33 (0·98)	1·67 (0·98)	1·47 (0·85)	2·12 (1·34) (p = 0·0014)	1·73 (1·28)	1·52 (0·99)	1·42 (1·04)
	Day 5	1·58 (1·05)	1·44 (0·94)	1·32 (0·73)	1·22 (0·55)	1·29 (0·74)	1·22 (0·62)	1·47 (0·76)	1·45 (0·91)	2·01 (1·30) (p = 0·0012)	1·63 (1·09)	1·52 (1·05)	1·31 (0·84)

Data are mean (SD) of daily symptoms score. Each symptom was scored on a scale of 1 to 7 (1 = no discomfort; 7 = very severe discomfort). P-value was estimated using Wilcoxon Signed Rank test between gluten challenge day and placebo challenge day. Significant values (p <= 0·05) are highlighted in red.

Table S9: Change in % immune cell types in peripheral blood mononuclear cells in particpants receiving Nexvax2 150 µg or placebo

Study		**3-dose study**		**Study**	**16-dose study**			
Cohort/s		**1st, 2nd & 3rd**	**3rd**	**Cohort/s**	**1st & 2nd**	**1st**	**3rd (biopsy)**	**3rd (biopsy)**
Treatment		**Placebo**	**Nexvax2**	**Treatment**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**
Dose			**150 µg**	**Dose**		**150 µg**		**150 µg**
N		**11**	**8**	**N**	**4**	**8**	**7**	**7**
Cell Type	**Day**			**Day**				
CD4+ T cells	Screening day 1	1·60 (5·64)	3·60 (4·41)	Screening day 1	-1·75 (2·17)	1·77 (8·42)	1·59 (5·96)	2·44 (3·45)
	Screening day 13	-0·25 (7·27)	5·02 (4·91)	Screening day 13	-3·63 (5·89)	0·07 (5·23)	-0·69 (5·80)	-0·83 (5·60)
	Day 1 baseline	47·56 (7·95)	50·83 (12·38)	Day 1 baseline	49·67 (8·87)	45·02 (10·22)	48·84 (10·10)	50·80 (5·35)
	Day 8	4·67 (12·52)	1·94 (4·23)	Day 8	2·05 (5·59)	-2·58 (6·24)	0·97 (5·87)	-1·17 (4·18)
	Day 15 EOT	1·03 (8·66)	2·89 (4·81)	Day 25	-1·60 (3·46)	0·65 (5·87)	1·08 (3·71)	-1·94 (4·42)
	Day 28	-0·78 (4·76)	3·43 (2·43)	Day 39	-1·27 (4·34)	-0·25 (5·50)	0·20 (8·15)	-0·97 (3·39)
	Day 47 EOS	4·60 (10·99)	4·88 (3·80)	Day 53 EOT	-0·03 (3·65)	-1·85 (6·29)	0·80 (5·53)	-3·19 (3·59)
	-			Day 66	0·35 (4·02)	-0·95 (4·99)	0·74 (4·22)	-2·86 (5·16)
	-			Day 92 EOS	3·75 (4·42)	-1·87 (5·75)	-2·13 (6·83)	-0·66 (3·48)
CD8+ T cells	Screening day 1	-2·13 (5·92)	-2·51 (4·26)	Screening day 1	-0·37 (3·80)	-3·75 (3·60)	-0·89 (3·20)	-1·74 (2·86)

	Screening day 13	-3·45 (6·63)	-0·80 (2·68)	Screening day 13	-0·12 (2·30)	-1·32 (1·95)	1·51 (1·14)	-0·10 (2·37)
	Day 1 baseline	28·51 (8·02)	24·09 (10·28)	Day 1 baseline	28·90 (12·37)	28·40 (13·61)	26·00 (8·55)	24·21 (6·13)
	Day 8	-3·24 (6·55)	-0·91 (2·14)	Day 8	-1·07 (0·95)	-0·82 (3·34)	1·41 (3·24)	-0·96 (1·99)
	Day 15 EOT	-1·61 (4·56)	-2·04 (5·00)	Day 25	1·92 (1·45)	-1·02 (2·27)	0·77 (1·87)	0·01 (0·99)
	Day 28	0·30 (2·67)	0·11 (3·06)	Day 39	1·65 (1·53)	-0·20 (3·84)	1·19 (3·28)	0·30 (2·71)
	Day 47 EOS	-3·77 (7·36)	-1·97 (4·32)	Day 53 EOT	1·05 (2·22)	-1·57 (4·37)	1·47 (3·39)	0·46 (2·65)
	-			Day 66	-0·23 (1·57)	2·17 (4·37)	0·77 (2·01)	-0·11 (1·46)
	-			Day 92 EOS	-0·90 (2·64)	0·92 (3·43)	1·09 (3·57)	-0·59 (1·40)
B cells	Screening day 1	-0·64 (1·95)	-0·34 (4·60)	Screening day 1	-1·40 (3·76)	-2·57 (4·25)	1·19 (2·09)	0·20 (2·96)
	Screening day 13	2·50 (4·68)	-0·67 (3·75)	Screening day 13	-2·83 (1·76)	-4·13 (5·34)	-0·53 (2·42)	-0·07 (3·39)
	Day 1 baseline	11·60 (5·96)	12·23 (6·56)	Day 1 baseline	10·98 (4·93)	17·43 (6·77)	11·39 (4·32)	11·17 (2·81)
	Day 8	-0·10 (2·38)	-0·57 (3·31)	Day 8	-1·62 (2·49)	-0·15 (5·05)	-1·91 (2·67)	0·06 (3·52)
	Day 15 EOT	0·74 (4·29)	-1·03 (3·24)	Day 25	-2·22 (2·53)	-1·17 (2·46)	-0·48 (1·78)	1·27 (5·19)
	Day 28	-0·05 (3·26)	-2·94 (3·70)	Day 39	-0·98 (1·66)	-2·45 (5·16)	0·49 (1·76)	0·47 (1·69)
	Day 47 EOS	-0·82 (1·07)	-1·55 (4·09)	Day 53 EOT	-2·95 (2·59)	0·67 (4·89)	0·19 (2·59)	0·97 (3·90)
	-			Day 66	-0·63 (1·71)	-5·10 (7·06)	0·10 (1·08)	0·41 (3·48)
	-			Day 92 EOS	-4·10 (3·58)	-3·35 (6·49)	-0·99 (2·46)	0·50 (1·39)

NK cells	Screening day 1	1·60 (3·22)	-0·34 (3·28)	Screening day 1	3·50 (5·64)	3·17 (6·86)	-1·93 (4·12)	0·29 (2·60)
	Screening day 13	1·92 (2·21)	-1·77 (4·95)	Screening day 13	4·45 (4·73)	3·33 (7·71)	-0·23 (4·31)	-0·10 (2·56)
	Day 1 baseline	9·37 (3·71)	10·87 (6·52)	Day 1 baseline	9·88 (4·61)	7·97 (3·38)	11·61 (5·22)	10·19 (2·69)
	Day 8	-0·40 (3·10)	-0·16 (2·34)	Day 8	0·07 (3·42)	2·67 (6·89)	-0·90 (3·30)	1·49 (4·54)
	Day 15 EOT	-0·01 (1·85)	0·16 (2·84)	Day 25	1·27 (2·97)	1·52 (4·98)	-1·70 (1·91)	1·13 (3·95)
	Day 28	0·78 (3·15)	-0·40 (2·75)	Day 39	-0·23 (1·56)	1·35 (3·51)	-1·70 (4·33)	-0·19 (2·03)
	Day 47 EOS	0·68 (3·06)	-0·82 (2·72)	Day 53 EOT	1·50 (2·91)	1·12 (3·56)	-1·93 (3·89)	1·03 (3·20)
	-			Day 66	0·60 (2·89)	2·22 (5·50)	-2·00 (2·42)	1·71 (2·42)
	-			Day 92 EOS	-0·18 (1·77)	4·28 (9·24)	1·11 (3·71)	-0·33 (3·29)

Data are mean (SD) for Day 1 of treatment (shaded in grey), and change in the % of cells from Day 1 of treatment on other days. P-value was estimated by Wilcoxon Sign Rank test between day 1 of treatment and other days. None of the p-values were significant ($p<=0.05$).

Table S10. Celiac disease-specific serology

CeD Serology	Study	3-dose study						16-dose study					
	Cohort/s	**1st, 2nd & 3rd**	**1st**	**2nd**	**3rd**	**4th (biopsy)**	**4th (biopsy)**	**1st**	**1st**	**2nd**	**2nd**	**3rd (biopsy)**	**3rd (biopsy)**
	Treatment	**Placebo**	**Nexvax2**	**Nexvax2**	**Nexvax2**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**	**Placebo**	**Nexvax2**
	Dose		**60µg**	**90µg**	**150µg**		**150µg**		**150 µg**		**300 µg**		**150 µg**
	Screening OGC	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**No**
	N	**11**	**8**	**8**	**8**	**3**	**2**	**4**	**8**	**3**	**8**	**7**	**7**
Tissue transglutaminase (tTG) IgA	Screening day 1	1 (9%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	1 (14%)
	End of Treatment	2 (18%)	1 (13%)	0 (0%)	2 (25%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	1 (14%)
Deamidated gliadin peptide (DGP) IgG	Screening day 1	1 (9%)	1 (13%)	0 (0%)	0 (0%)	1 (33%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (14%)
	End of Treatment	4 (36%)	1 (13%)	2 (25%)	2 (25%)	1 (33%)	0 (0%)	2 (50%)	1 (13%)	0 (0%)	1 (13%)	1 (14%)	2 (29%)
Deamidated gliadin peptide (DGP) IgA	Screening day 1	2 (18%)	3 (38%)	2 (25%)	2 (25%)	0 (0%)	0 (0%)	2 (50%)	1 (13%)	0 (0%)	2 (25%)	0 (0%)	2 (29%)
	End of Treatment	4 (36%)	2 (25%)	2 (25%)	2 (25%)	0 (0%)	0 (0%)	2 (50%)	1 (13%)	0 (0%)	2 (25%)	1 (14%)	2 (29%)

Data are n (%) indicating number of participants with elevated serology; Normal serology was defined as: tTG IgA upper level of normal is <4·0; DGP IgG upper level of normal is <20; DGP IgA upper level of normal is <20

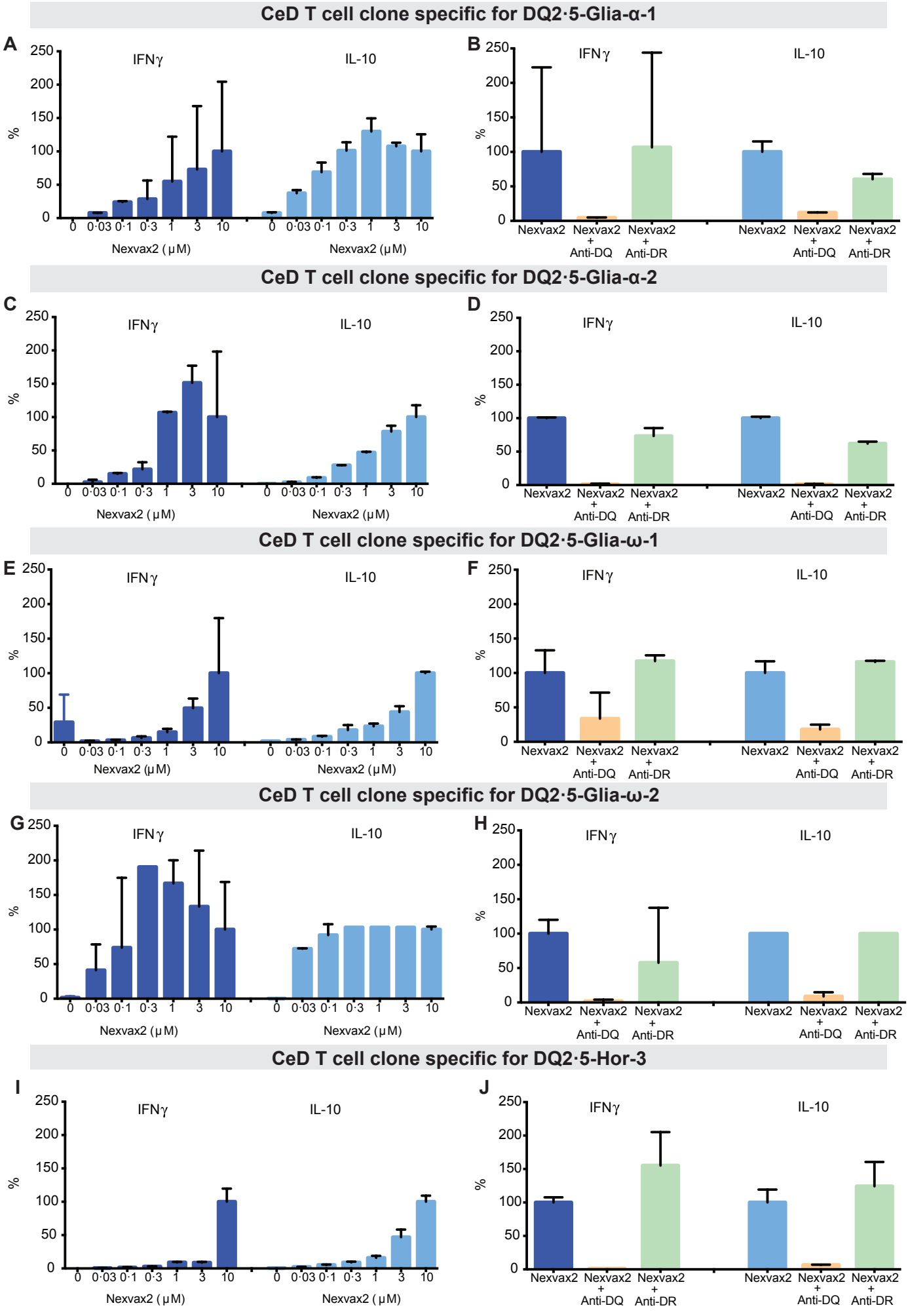
Table S11. Duodenal quantitative histology

Treatment	N	Timing	Sites of biopsy	Modified Marsh types	Villous height to crypt depth ratio	Intraepithelial lymphocyte density
				Median (min-max)	Median (IQR)	Median (IQR)
3-dose study						
Nexvax2 150 µg	2	Screening period	2nd and 3rd parts	0 (0 - 0)	2.04 (0.69)	21 (18)
	2	Post-treatment period		0 (0 - 0)	2.49 (0·67)	21 (10)
	2	Screening period	Bulb and 1st part	0 (0 - 0)	1.52 (0·64)	13 (18)
	2	Post-treatment period		0 (0 - 0)	1.45 (0·01)	17 (8)
Placebo	3	Screening period	2nd and 3rd parts	0 (0 - 1)	1.75 (0.62)	45 (21)
	3	Post-treatment period		1 (1 – 3a)	2.09 (.71)	36 (6)
	3	Screening period	Bulb and 1st part	0 (0 - 1)	1.43 (0·32)	46 (18)
	3	Post-treatment period		0 (0 - 1)	1·53 (0·28)	42 (8)
16-dose study						
Nexvax2 150 µg	7	Screening period	2nd and 3rd parts	0 (0 - 3c)	1·74 (0·54)	46 (24)
	7	Post-treatment period		0 (0 - 3c)	1·56 (0·58)	51 (33)
	7	Screening period	Bulb and 1st part	0 (0 - 3a)	1·44 (0·46)	30 (29)
	7	Post-treatment period		0 (0 - 3a)	1·70 (0·47)	36 (15)
Placebo	7	Screening period	2nd and 3rd parts	0 (0 - 1)	2·10 (0·25)	35 (16)
	7	Post-treatment period		0 (0 - 3b)	1·92 (0·35)	32 (18)
	7	Screening period	Bulb and 1st part	0 (0 - 1)	1·69 (0·48)	28 (13)
	7	Post-treatment period		0 (0 - 3a)	1·65 (0·28)	35 (18)

Table S12. Fold-change in plasma complement cytokines at 6h post-dose

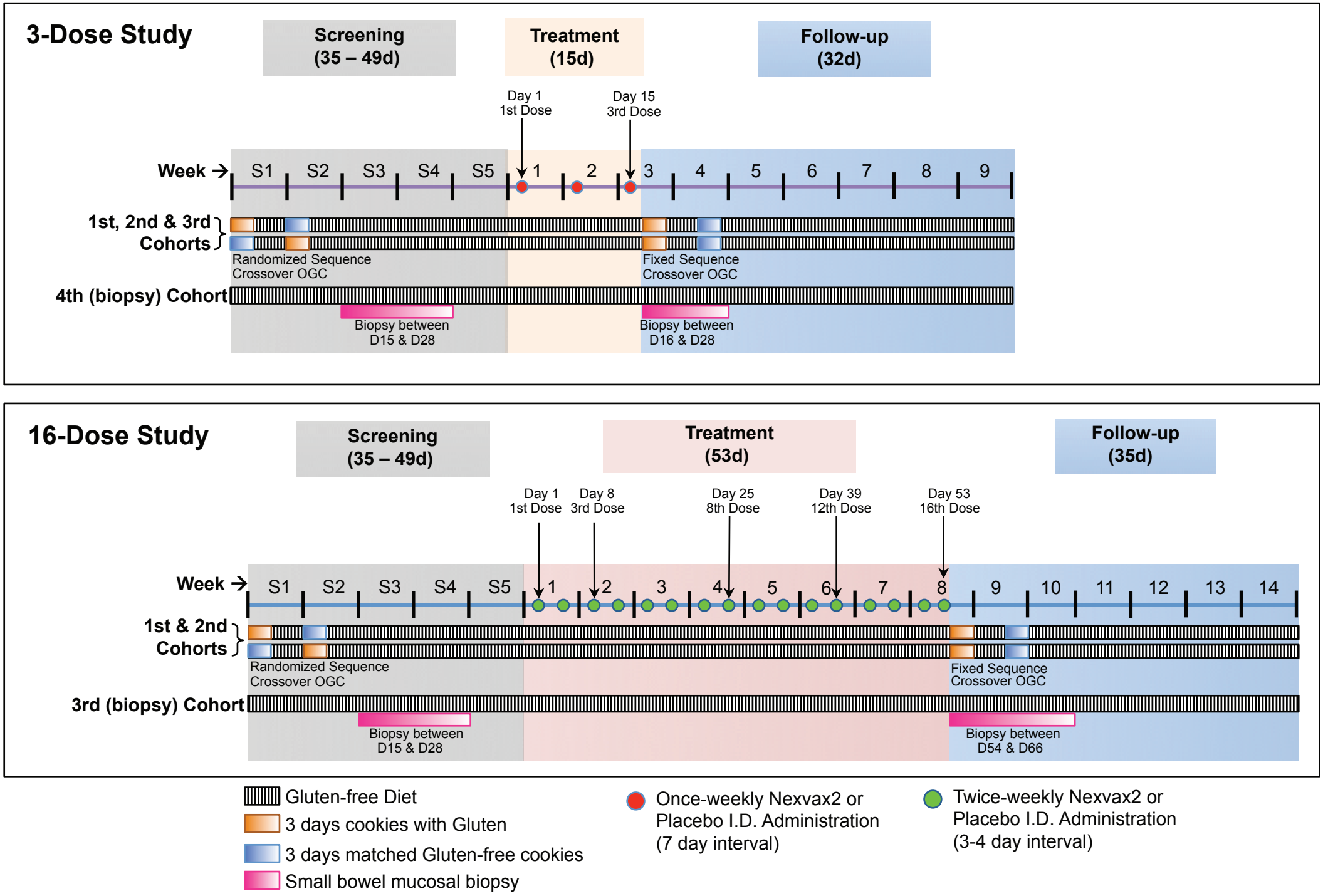
Cohort	1st & 2nd				1st				3rd (biopsy)				3rd (biopsy)			
Treatment	Placebo				Nexvax2 150ug				Placebo				Nexvax2 150ug			
N	7				8				7				7			
Dose	First Dose		Last Dose		First Dose		Last Dose		First Dose		Last Dose		First Dose		Last Dose	
Cytokines	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value	Fold change	P-value
C" C2	0.92 (0.07)	0.2500	0.95 (0.12)	0.8750	0.95 (0.11)	0.6731	0.87 (0.11)	0.4368	0.92 (0.10)	0.2388	0.91 (0.10)	0.4377	0.95 (0.03)	0.2190	0.94 (0.04)	0.4377
C" C4b	1.00 (0.05)	1.0000	0.98 (0.04)	0.8750	0.97 (0.05)	0.5569	0.97 (0.06)	0.7291	0.97 (0.05)	0.5569	0.98 (0.04)	0.8750	0.99 (0.04)	0.8922	0.99 (0.06)	0.8427
C" C5	1.00 (0.04)	0.8922	0.98 (0.05)	0.8427	0.98 (0.05)	0.6496	0.96 (0.10)	0.8750	0.97 (0.07)	0.6158	0.99 (0.06)	0.9681	0.99 (0.04)	0.6158	1.00 (0.03)	0.8750
C" C5a	0.99 (0.03)	0.6496	1.00 (0.06)	0.9800	0.99 (0.03)	0.6250	0.96 (0.06)	0.7291	0.97 (0.04)	0.6158	0.99 (0.02)	0.7158	0.99 (0.04)	0.8922	1.00 (0.02)	1.0000
C" C9	0.99 (0.08)	0.8370	1.02 (0.07)	0.9800	1.05 (0.15)	0.7470	1.04 (0.13)	0.8750	1.03 (0.14)	0.9087	1.01 (0.05)	0.8750	0.96 (0.08)	0.6731	1.05 (0.13)	0.8750
C" FD	0.94 (0.08)	0.3604	0.88 (0.10)	0.7000	0.89 (0.12)	0.2190	0.92 (0.14)	0.7291	0.91 (0.10)	0.2190	0.89 (0.10)	0.4377	0.92 (0.09)	0.2190	0.92 (0.06)	0.4368
MBL	0.98 (0.07)	0.8370	0.97 (0.06)	0.8427	0.94 (0.06)	0.2190	0.92 (0.12)	0.8427	0.95 (0.10)	0.8370	0.96 (0.05)	0.7658	0.99 (0.03)	0.2734	1.00 (0.04)	0.8750
C" FI	1.00 (0.03)	0.8922	0.98 (0.07)	0.8750	0.94 (0.07)	0.2734	0.94 (0.08)	0.4377	0.96 (0.06)	0.2190	0.99 (0.06)	0.8750	0.98 (0.04)	0.6731	0.99 (0.04)	0.8750
C" C1q	0.99 (0.06)	0.7218	0.98 (0.06)	0.8750	0.96 (0.06)	0.4863	0.94 (0.09)	0.7000	0.95 (0.08)	0.2190	0.97 (0.07)	0.7828	0.99 (0.04)	0.8370	0.98 (0.07)	0.8750
C" C3	1.05 (0.26)	1.0000	1.03 (0.41)	1.0000	0.94 (0.15)	0.6496	0.93 (0.17)	0.7291	0.83 (0.18)	0.2190	0.95 (0.18)	0.8427	0.85 (0.13)	0.2500	0.96 (0.21)	0.8333
C" C4	1.01 (0.07)	0.8922	0.95 (0.04)	0.7000	0.95 (0.05)	0.2190	0.96 (0.11)	0.9035	0.96 (0.09)	0.6158	0.99 (0.08)	0.8750	0.98 (0.06)	0.6496	0.99 (0.06)	0.9906
C" FB	1.01 (0.06)	0.9722	0.95 (0.04)	0.7778	0.95 (0.05)	0.2500	0.95 (0.11)	0.8750	0.95 (0.10)	0.5569	0.99 (0.09)	0.9800	0.98 (0.06)	0.6731	1.00 (0.06)	0.9906
C" FH	1.03 (0.08)	0.6731	0.95 (0.04)	0.7000	0.95 (0.06)	0.2190	0.96 (0.10)	0.9035	0.96 (0.09)	0.5569	0.98 (0.09)	0.8427	0.97 (0.07)	0.6496	1.00 (0.07)	0.9906
Properdin	1.01 (0.05)	0.8370	0.96 (0.04)	0.7778	0.98 (0.05)	0.6158	0.95 (0.11)	0.8333	0.96 (0.10)	0.6692	0.98 (0.08)	0.8750	0.99 (0.06)	0.9410	1.00 (0.06)	1.0000

Data are mean (SD) for paired fold change. Paired fold-change was estimate at 6h post-dose compared to pre-dose concentration levels. P-value was estimated by Wilcoxon Sign Rank test of pre-dose and 6h post-dose concentrations. FDR-adjusted p-values, were estimated using Benjamini-Hochberg method.



Supp. Figure S1: Nexvax2 peptides stimulate IFNγ and IL-10 secretion by T-cell clones from HLA-DQ2.5+ CeD donors that are specific for immunodominant, HLA-DQ2.5-restricted gluten epitopes. Cytokine concentrations measured by multiplex bead assay in media after 24 h incubation of T cell clones with HLA-DQ2.5+ B cell lines and equimolar concentrations of the three peptides in Nexvax2. Cytokine levels are represented as percent of concentrations stimulated by Nexvax2 with each **(continued)**

(Supp. Figure S1 continued) constituent peptide at 10 μM. T cell clones were specific for one of five HLA-DQ2.5-restricted gluten epitopes present in Nexvax2 peptides shown in Table S1 (A, C, E, G, and I). Consistent with gluten-reactive T cell clones being activated by Nexvax2 peptides bound to HLA-DQ2.5, cytokine secretion was inhibited by co-incubation with anti-HLA-DQ antibody (clone SPvL3), but not anti-HLA-DR (clone L243) at 10 μg/mL with Nexvax2 peptides at 10 μM (B, D, F, H, J).

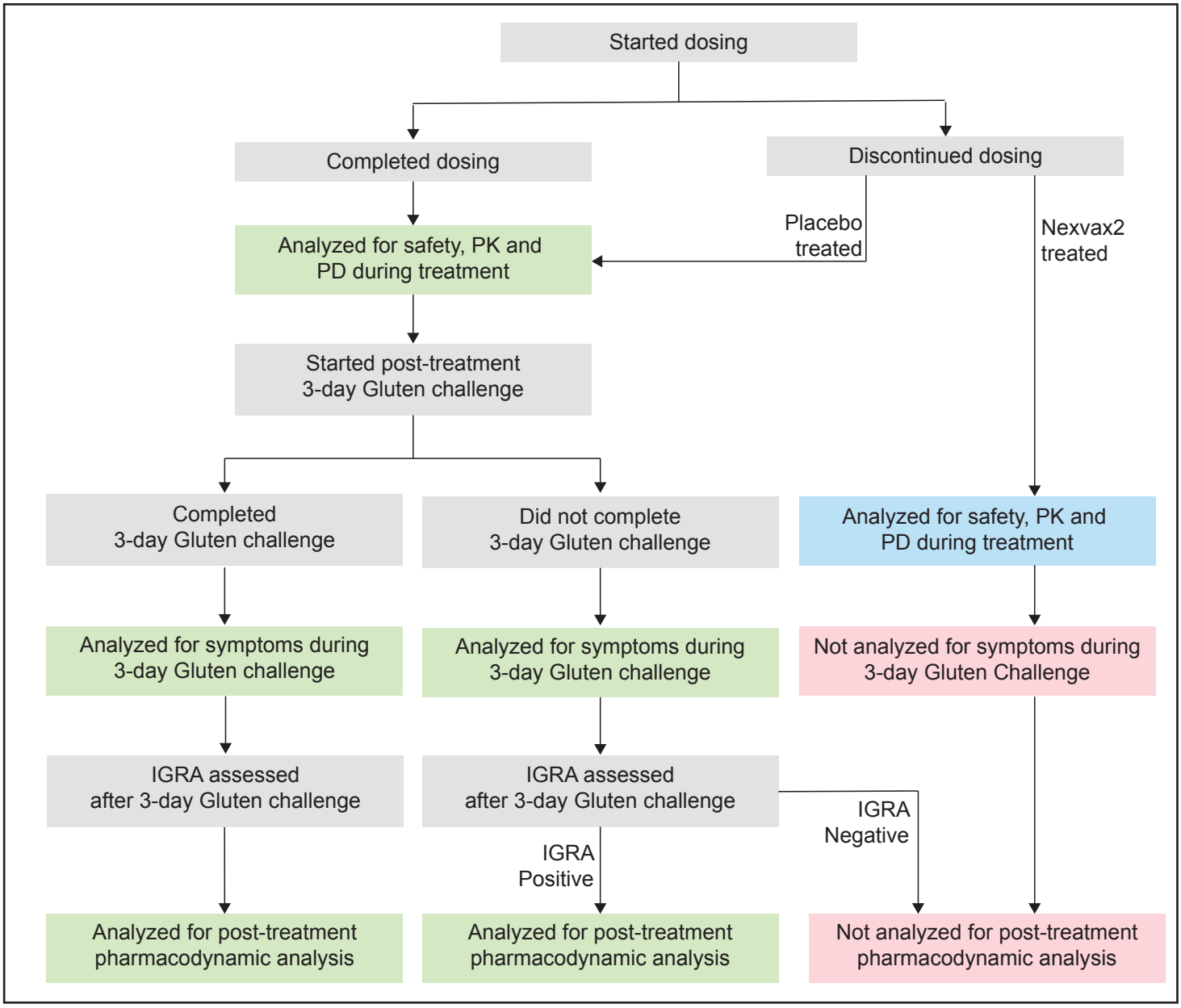


Supp. Figure S2: Study designs

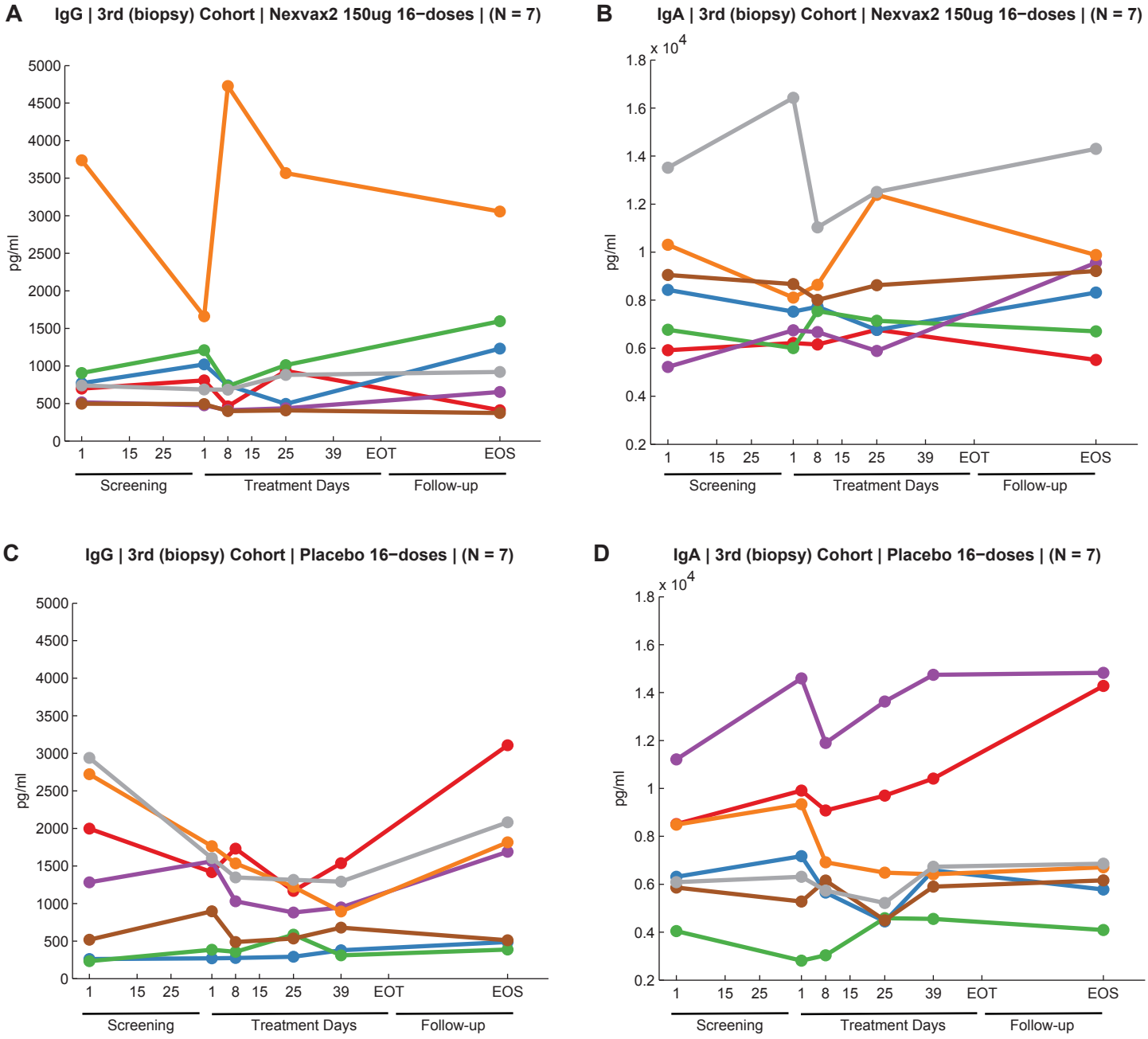
A Gluten cookies

B Placebo cookies

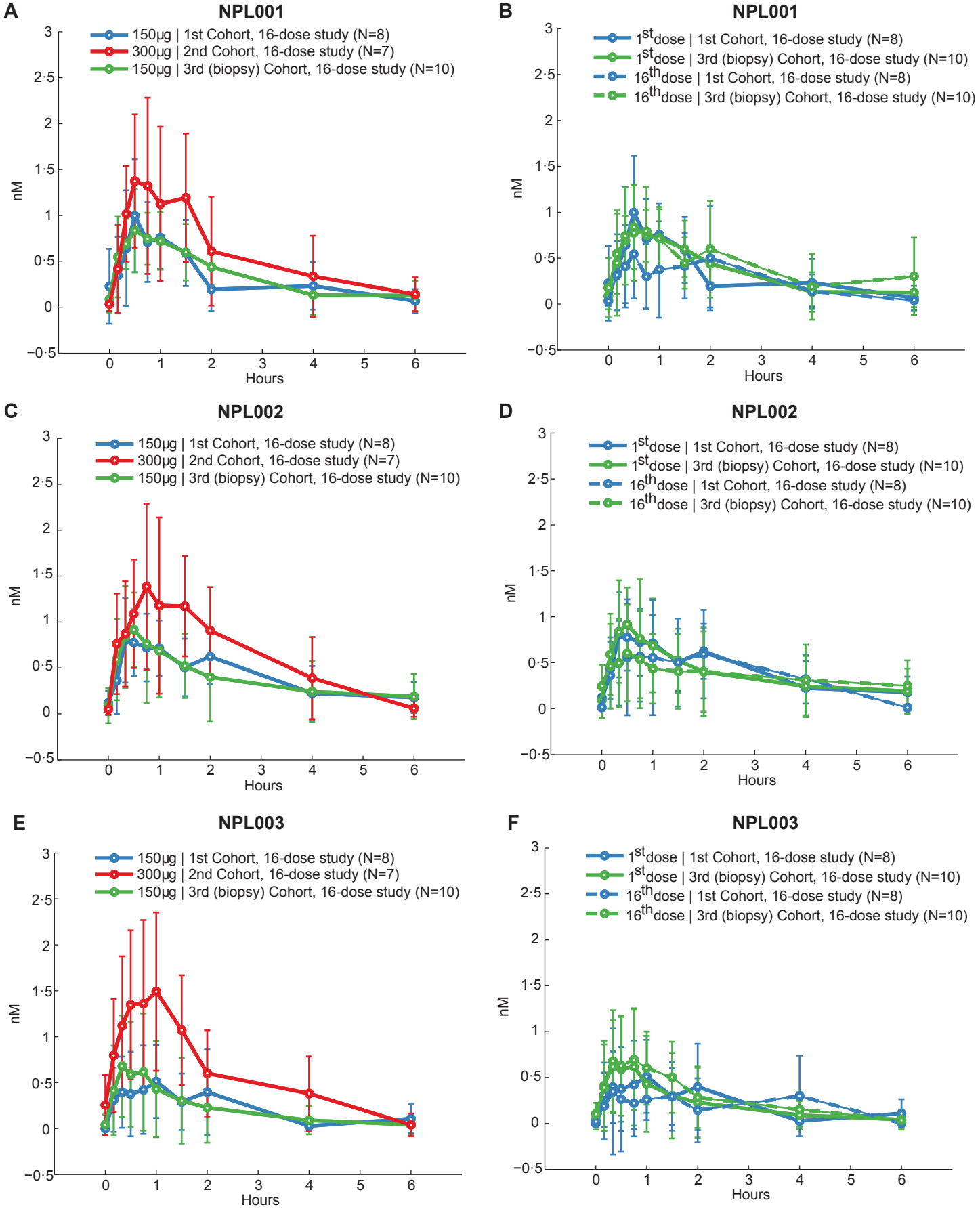
Supp. Figure S3: Cookies used for placebo-controlled, crossover oral gluten challenges. Gluten-containing (A), and matched gluten-free cookies (B) are shown. The gluten-containing cookies were prepared using a mixture of wheat, barley and rye flour providing a total of approximately 3 g gluten according to the Osborne calculation. Gluten-free cookies were matched for their appearance, weight, taste and consistency. Gluten-free cookies had no detectable gluten by R5 ELISA. Participants were advised to eat each cookie slowly over a 1-1.5 hour interval, and eat all three cookies completely each day, and to continue their usual gluten-free diet driven by their individual appetite on these three days.



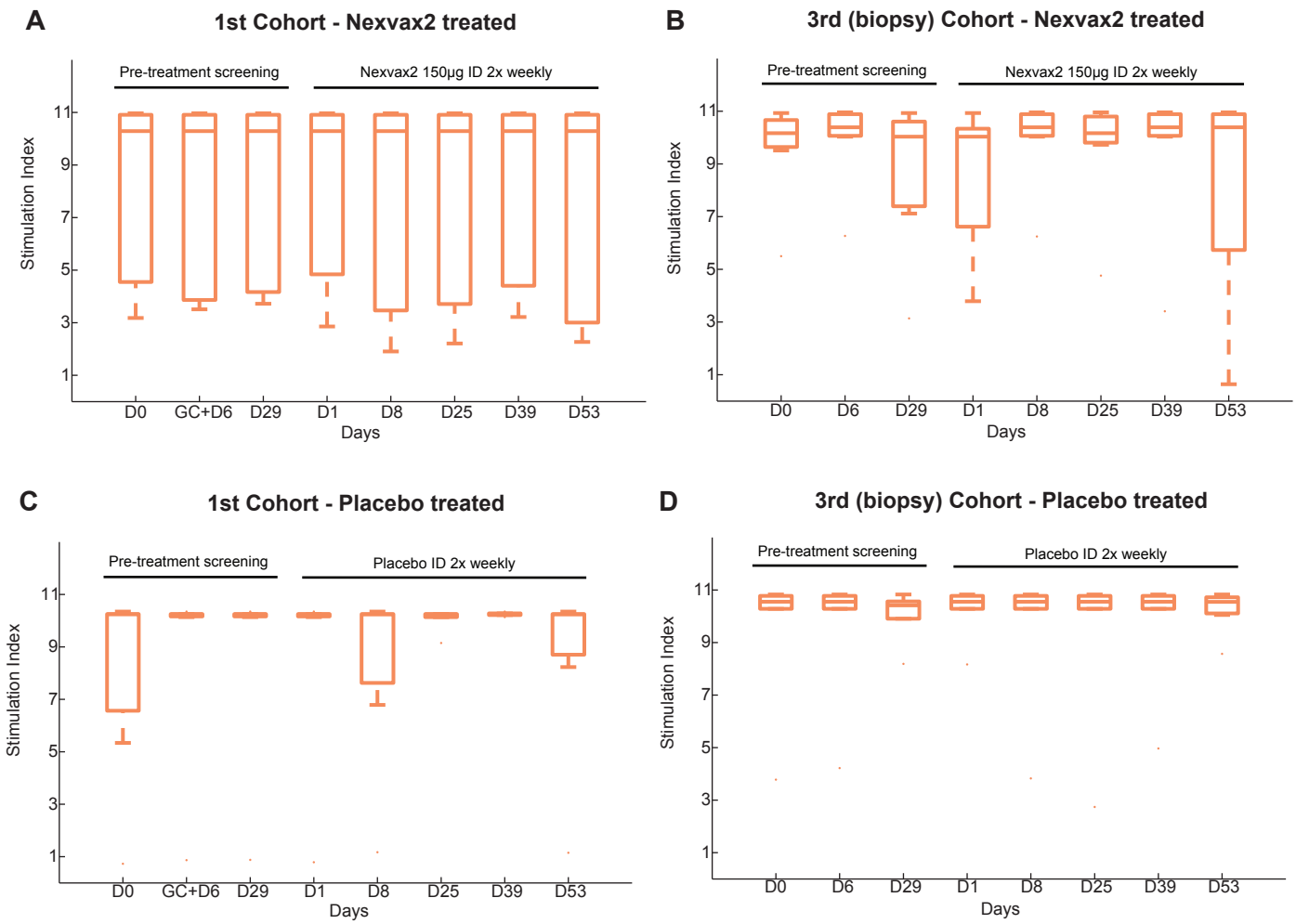
Supp. Figure S4: Participant flow map and data analysis populations for ascending dose cohorts



Supp. Figure S5: Anti-Nexvax2 antibodies. Anti-therapeutic IgG and IgA specific for Nexvax2 peptides were tested by ELISA in serum. IgG and IgA levels are shown for the biopsy cohort in the 16-dose study at Screening day 1, pre-dose on Treatment day 1 and before the 3rd, 8th, 12th dose and at the end-of-study.



Supp. Figure S6: Pharmacokinetics of Nexvax2 peptides in plasma. Mean (±SEM) plasma concentrations of NPL001 (A), NPL002 (C), and NPL003 (E) are shown after the first dose of Nexvax2 150µg (1st Cohort) or 300µg (2nd Cohort) in ascending dose cohorts that had OGC in screening, and in the biopsy cohort (3rd [biopsy] Cohort) in 16-dose Study. Mean (±SEM) plasma concentrations of NPL001 (B), NPL002 (D), and NPL003 (F) after the first and last dose of Nexvax2 150µg for 1st Cohort and 3rd (biopsy) Cohort in 16-dose Study. Measured concentrations of NPL001, NPL002, and NPL003 were below the lower levels of quantitation (2.6 nM, 5.5 nM, and 5.3 nM, respectively), but frequently above the lower levels of detection (0.05 nM, 0.1 nM, and 0.4 nM, respectively).



Supp. Figure S7: CEF activation of T cells ex vivo in blood. Fold increase (stimulation index) in IFNγ release by whole blood (IGRA) incubated for 24h with CEF peptides compared to negative control during screening, and treatment periods in the 16-dose study for participants receiving Nexvax2 150 µg in the 1st cohort (A) that had OGC during screening, and in the 3rd (biopsy) cohort (B), or who received placebo in the 1st cohort (C), and in the 3rd (biopsy) cohort (D). Median with interquartile range are shown. The CEF peptide pool contains MHC Class I epitopes commonly recognized by memory CD8+ T cells specific for cytomegalovirus, Epstein-Barr virus, or influenza antigens.