EBioMedicine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Epitope-Specific Immunotherapy Targeting CD4-Positive T Cells in Celiac Disease: Safety, Pharmacokinetics, and Effects on Intestinal Histology and Plasma Cytokines with Escalating Dose Regimens of Nexvax2 in a Randomized, Double-Blind, Placebo-Controlled Phase 1 Study

A. James M. Daveson [a], Hooi C. Ee [b], Jane M. Andrews [c], Timothy King [d], Kaela E. Goldstein [f], John L. Dzuris [f], James A. MacDougall [e], Leslie J. Williams [f], Anita Treohan [f], Michael P. Cooreman [f], Robert P. Anderson [f,*]

[a] Faculty of Medicine, University of Queensland, QLD, Australia
[b] Department of Gastroenterology, Sir Charles Gairdner Hospital, WA, Australia
[c] Department of Gastroenterology and Hepatology, Royal Adelaide Hospital, SA, Australia
[d] Department of Gastroenterology, Auckland City Hospital, Auckland, New Zealand
[e] PROMETRIKA, LLC, Cambridge, MA, USA
[f] ImmusanT Inc., Cambridge, MA, USA

ARTICLE INFO

Article history:
Received 14 November 2017
Accepted 20 November 2017
Available online xxxx

Keywords:
Celiac disease
Nexvax2
Immunotherapy
T cell
Peptide
Randomized controlled trial

ABSTRACT

Background: Nexvax2® is a novel, peptide-based, epitope-specific immunotherapy intended to be administered by regular injections at dose levels that increase the threshold for clinical reactivity to natural exposure to gluten and ultimately restore tolerance to gluten in patients with celiac disease. Celiac disease patients administered fixed intradermal doses of Nexvax2 become unresponsive to the HLA-DQ2·5-restricted gluten epitopes in Nexvax2, but gastrointestinal symptoms and cytokine release mimicking gluten exposure, that accompany the first dose, limit the maximum tolerated dose to 150 μg. Our aim was to test whether stepwise dose escalation attenuated the first dose effect of Nexvax2 in celiac disease patients.

Methods: We conducted a randomized, double-blind, placebo-controlled trial at four community sites in Australia (3) and New Zealand (1) in HLA-DQ2·5 genotype positive adults with celiac disease who were on a gluten-free diet. Participants were assigned to cohort 1 if they were HLA-DQ2·5 homozygotes; other participants were assigned to cohort 2, or to cohort 3 subsequent to completion of cohort 2. Manual central randomization without blocking was used to assign treatment for each cohort. Initially, Nexvax2-treated participants in cohorts 1 and 2 received an intradermal dose of 30 μg (consisting of 10 μg of each constituent peptide), followed by 60 μg, 90 μg, 150 μg, and then eight doses of 300 μg over six weeks, but this was amended to include doses of 3 μg and 9 μg and extended over a total of seven weeks. Nexvax2-treated participants in cohort 3 received doses of 3 μg, 9 μg, 30 μg, 60 μg, 90 μg, 150 μg, 300 μg, 450 μg, 600 μg, 750 μg, and then eight of 900 μg over nine weeks. The dose interval was 3 or 4 days. Participants, care providers, data managers, sponsor personnel, and study site personnel were blinded to treatment assignment. The primary outcome was the number of adverse events and percentage of participants with adverse events during the treatment period. This completed trial is registered with ClinicalTrials.gov, number NCT02528799.

Findings: From the 73 participants who we screened from 19 August 2015 to 31 October 2016, 24 did not meet eligibility criteria, and 36 were ultimately randomized and received study drug. For cohort 1, seven participants received Nexvax2 (two with the starting dose of 30 μg and then five at 3 μg) and three received placebo. For cohort 2, 10 participants received Nexvax2 (four with starting dose of 30 μg and then six at 3 μg) and four received placebo. For cohort 3, 10 participants received Nexvax2 and two received placebo. All 36 participants were included in safety and immune analyses, and 33 participants completed treatment and follow-up; in cohort 3, 11 participants were assessed and included in pharmacokinetics and duodenal histology analyses. Whereas the maximum dose of Nexvax2 had previously been limited by adverse events and cytokine release, no such effect was observed when dosing escalated from 3 μg up to 300 μg in HLA-DQ2·5 homozygotes or to 900 μg in HLA-DQ2.5 non-homozygotes. Adverse events with Nexvax2 treatment were less common in cohorts 1 and 2 with the starting dose of 3 μg (72 for 11 participants) than with the starting dose of 30 μg (91 for six participants). Adverse events during the treatment period in placebo-treated participants (46 for nine participants) were similar

* Corresponding author at: ImmusanT Inc., One Kendall Square, Suite B2004, Cambridge, MA 02139, USA.
E-mail address: bob@immusant.com (R.P. Anderson).

https://doi.org/10.1016/j.ebiom.2017.11.018

to those in Nexvax2-treated participants when the starting dose was 3 μg in cohort 1 (16 for five participants), cohort 2 (56 for six participants), and cohort 3 (44 for 10 participants). Two participants in cohort 2 and one in cohort 3 who received Nexvax2 starting at 3 μg did not report any adverse event, while the other 33 participants experienced at least one adverse event. One participant, who was in cohort 1, withdrew from the study due to adverse events, which included abdominal pain graded moderate or severe and associated with nausea after receiving the starting dose of 30 μg and one 60 μg dose. The most common treatment-emergent adverse events in the Nexvax2 participants were headache (52%), diarrhoea (48%), nausea (37%), abdominal pain (26%), and abdominal discomfort (19%). Administration of Nexvax2 at dose levels from 150 μg to 900 μg preceded by dose escalation was not associated with elevations in plasma cytokines at 4 h. Nexvax2 treatment was associated with trends towards improved duodenal histology. Plasma concentrations of Nexvax2 peptides were dose-dependent.

Interpretation: We show that antigenic peptides recognized by CD4-positive T cells in an autoimmune disease can be safely administered to patients at high maintenance dose levels without immune activation if preceded by gradual dose escalation. These findings facilitate efficacy studies that test high-dose epitope-specific immunotherapy in celiac disease.

1. Introduction

"Immune tolerance" has been defined as "a state of indifference or non-reactivity towards a substance that would normally be expected to excite an immunological response" (Medawar, 1961). In patients with celiac disease, immunological tolerance to dietary gluten is replaced by a T cell-mediated hypersensitivity reaction that results in small intestinal injury and digestive symptoms (Sollid and Jabri, 2013). Quarantining the immune system with a life-long, strict, gluten-free diet is currently the mainstay of management for celiac disease (Ludvigsson et al., 2014). Gluten-free diet for six months or more usually results in normalisation of serum antibodies specific for gluten-derived peptides and autoantibodies specific for transglutaminase, but signs of ongoing intestinal injury persist in many patients (Ludvigsson et al., 2014). Recurrent digestive symptoms on gluten-free diet are common, and the risk of acute symptoms that can follow within hours of accidental gluten exposure is ever present (See et al., 2015). The shortcomings of a gluten-free diet highlight a substantial unmet need that is being addressed by clinical development of agents that may enhance the effectiveness of dietary therapy (Kurada et al., 2016). However, overcoming the gluten-specific adaptive immune response and ultimately restoring immune tolerance without global immunosuppression is the long-term goal of pharmacotherapy for autoimmune diseases, including celiac disease (Sabatos-Peyton et al., 2010).

Antigen-specific CD4-positive T cells are implicated in many human autoimmune diseases that have strong associations with genes in the class II region of the major histocompatibility complex. Celiac disease stands out as a candidate for development of peptide-based immunotherapy because 90% of patients carry genes encoding human leukocyte antigen (HLA)-DQ2·5 (*HLA-DQA1*05* and *HLA-DQB1*02*) and there is clear understanding of the antigenic peptides (epitopes) recognized by disease-associated (gluten-specific) CD4-positive T cells (Karell et al., 2003; Anderson and Jabri, 2013; Tye-Din et al., 2010). Following systemic peptide administrations, CD4-positive T cells repeatedly exposed to the epitope recognized by their T cell antigen receptor become unresponsive to further antigenic stimulation and may be deleted, rendered anergic or adopt regulatory properties (Pape et al., 1998; McPherson et al., 2014). Therefore, short, soluble peptides containing epitopes for CD4-positive T cells that cause autoimmune disease could have the potential to control or abort destructive autoreactivity (Larche and Wraith, 2005).

Early-stage clinical trials of adjuvant-free peptide immunotherapies for multiple sclerosis and type-1 diabetes have shown promising clinical results, but dose selection in clinical trials has been empirical (Streeter et al., 2015; Alhadj et al., 2017). Nexvax2® is a peptide-based, epitope-specific immunotherapy under development for celiac disease (Goel et al., 2017). Nexvax2 is customized for HLA-DQ2·5-positive patients and is a simple mixture of three synthetic peptides dissolved in normal saline that is administered by intradermal injection. The peptides in Nexvax2 (NPL001, NPL002, and NPL003) each contains 15 or 16 amino acids and encompass at least five HLA-DQ2.5-restricted epitopes frequently recognized by gluten-reactive CD4 positive T cells (Goel et al., 2017; Tye-Din et al., 2010). Phase 1 clinical trials of Nexvax2 in celiac disease have provided insight into the safety and therapeutic potential of peptide immunotherapy as well as the immunological effects of small antigenic peptides that are recognized by a discrete population of gut-homing or gut-located, memory CD4 positive T cells (Ráki et al., 2007). As well as having the potential to modify gluten-specific immune responses, injections of Nexvax2 are effectively systemic "gluten epitope challenges" that test the responsiveness of gluten-reactive CD4 positive T cells specific for epitopes in Nexvax2.

Previous phase 1b studies have assessed the safety, tolerability, and bioactivity of Nexvax2 in HLA-DQ2·5 positive participants with celiac disease following a gluten-free diet (Brown et al., 2011; Goel et al., 2017); Nexvax2 has been assessed in fixed, repeat doses from 9 μg to 300 μg for up to eight weeks with dose intervals as short as 3 to 4 days. A course of intradermal injections with Nexvax2, particularly at the highest tolerated dose of 150 μg, results in unresponsiveness to the gluten epitopes in Nexvax2, but the first administration at dose levels above 30 μg has sometimes been associated with clinical symptoms similar to those experienced by patients with celiac disease on a gluten-free diet when they consume gluten (Brown et al., 2011; Goel et al., 2017). Participants in these phase 1 studies who were HLA-DQ2·5 homozygotes and had completed a 3-day gluten challenge a month before dosing were particularly susceptible to acute gastrointestinal symptoms, which occurred 2 to 5 h after the first administration of Nexvax2. The first dose of Nexvax2 also caused immune activation as early as 2 h, which was demonstrated by elevations in plasma interleukin (IL)-2, IL-6, IL-10, monocyte chemoattractant protein-1 (MCP-1 or CCL2), interferon gamma-induced protein 10 (IP-10 or CXCL10), and IL-8 that peaked at 4 to 6 h (Goel et al., 2016). These features have not been observed with the first dose of antigenic peptides in multiple sclerosis or type-1 diabetes (Streeter et al., 2015; Alhadj et al., 2017), but bare some similarities to the first dose effects of immunosuppressive biologics that initially activate T cells (Chatenoud et al., 1990), and also to the timing of isolated late asthmatic reaction elicited by T-cell stimulatory allergen-derived peptide (Haselden et al., 1999).

Although higher maintenance dose levels of peptide immunotherapy are hypothesized to be more efficacious (Sabatos-Peyton et al., 2010), adverse events associated with the first dose of Nexvax2 prevented further evaluation of Nexvax2 at levels of 300 μg or higher in fixed dose regimens (Goel et al., 2017). Studies in genetically modified mice with clonal T cell populations indicate that systemic cytokine release caused by CD4-positive T cell activation after subcutaneous

Please cite this article as: Daveson, A.J.M., et al., Epitope-Specific Immunotherapy Targeting CD4-Positive T Cells in Celiac Disease: Safety, Pharmacokinetics, and Effects on Intestin..., EBioMedicine (2017), https://doi.org/10.1016/j.ebiom.2017.11.018