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NDV-3, a Recombinant Alum-Adjuvanted Vaccine for *Candida* and *Staphylococcus aureus* is Safe and Immunogenic in Healthy Adults

Clint S. Schmidt^a, C. Jo White^b, Ashraf S. Ibrahim^{c,e,f}, Scott G. Filler^{c,e,f}, Yue Fu^{c,e,f}, Michael R. Yeaman^{c,d,e,f}, John E. Edwards Jr.^{c,e,f}, and John P. Hennessey Jr.^a

^aNovaDigm Therapeutics, Inc., 4201 James Ray Drive, Suite 2200, REAC 1 Building, Grand Forks ND 58202, USA

^bCJW Consulting, Ambler PA, USA

^cThe Division of Infectious Diseases, Department of Medicine, Harbor-University of California at Los Angeles (UCLA) Medical Center, 1000 West Carson St., Torrance CA 90502, USA

^dThe Division of Molecular Medicine, Department of Medicine, Harbor-University of California at Los Angeles (UCLA) Medical Center, 1000 West Carson St., Torrance CA 90502, USA

^eLos Angeles Biomedical Research Institute at Harbor-University of California at Los Angeles (UCLA) Medical Center, 1124 West Carson St. Torrance CA 90502 USA

^fThe David Geffen School of Medicine at UCLA, Los Angeles CA 90024 USA

Abstract

The investigational vaccine, NDV-3, contains the N-terminal portion of the *Candida albicans* agglutinin-like sequence 3 protein (Als3p) formulated with an aluminum hydroxide adjuvant in phosphate-buffered saline. Preclinical studies demonstrated that the Als3p vaccine antigen protects mice from oropharyngeal, vaginal and intravenous challenge with *C. albicans* and other selected species of *Candida* as well as both intravenous challenge and skin and soft tissue infection with *Staphylococcus aureus*. The objectives of this first-in-human Phase I clinical trial were to evaluate the safety, tolerability and immunogenicity of NDV-3 at two different antigen levels compared to a saline placebo. Forty healthy, adult subjects were randomized to receive one dose of NDV-3 containing either 30 or 300 µg of Als3p, or placebo. NDV-3 at both dose levels was safe and generally well-tolerated. Anti-Als3p total IgG and IgA1 levels for both doses reached peak levels by day 14 post vaccination, with 100% seroconversion of all vaccinated subjects. On average, NDV-3 stimulated peripheral blood mononuclear cell (PBMC) production of both IFN-γ and IL-17A, which peaked at day 7 for subjects receiving the 300 µg dose and at day 28 for those receiving the 30 µg dose. Six months after receiving the first dose of NDV-3, nineteen subjects

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Corresponding author: John P. Hennessey, Jr., Ph.D., 4201 James Ray Dr, Suite 2200, Grand Forks, ND 58202, 267-640-5189 (phone), 1-701-335-7121 (fax), john_hennessey@novadigm.net. Alternate corresponding author: Clint S. Schmidt, Ph.D., 4201 James Ray Drive, Suite 2200, Grand Forks, ND 58202, 701-757-5158, clint_schmidt@novadigm.net.

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Conflict of Interest

C.S.S., A.S.I., S.G.F., Y.F., M.R.Y., J.E.E. and J.P.H. own equity in NovaDigm Therapeutics, Inc., which is developing vaccine technologies.

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received a second dose of NDV-3 identical to their first dose to evaluate memory B- and T-cell immune responses. The second dose resulted in a significant boost of IgG and IgA1 titers in >70% of subjects, with the biggest impact in those receiving the 30 µg dose. A memory T-cell response was also noted for IFN- γ in almost all subjects and for IL-17A in the majority of subjects. These data support the continued investigation of NDV-3 as a vaccine candidate against *Candida* and *S. aureus* infections.

Keywords

vaccine; phase 1; first-in-human; Als3p; *Candida*; *Staphylococcus aureus*

Introduction

Candida sp. are a major cause of hospital acquired infections in the U.S. and worldwide [1, 2]. *Candida* sp. are now equivalent to enterococci as the third most frequent hospital acquired bloodstream isolates [3, 4], accounting for 10% of bloodstream infections and 11% of catheter-related infections [5]. Population-based surveys in the U.S. have reported the annual incidence of *Candida* bloodstream infections is approximately 20 cases per 100,000 people (60,000 cases per year) [6, 7]. In high risk/hospitalized patients, this incidence increases 50-fold [1, 6, 8, 9]. These rates represent 15 to 20-fold increases compared to two decades earlier [10–12]. In addition to hematogenously disseminated candidiasis, mucosal candidal infections are common and can be persistent in some patients, causing recurrent disease several times per year. Most notable in this respect is recurrent vulvovaginal candidiasis, which impacts 5–8% of women in the US [13].

Staphylococcus aureus is the most common cause of skin and skin structure infections [14] and endocarditis [5], and the second most common cause of bacteremia [3, 4]. *S. aureus* is a primary cause of a variety of hospital acquired infections, including ventilator-associated pneumonia, intravenous-catheter associated infections, post-surgical wound infections and is also a predominant cause of battlefield wound infections [15, 16]. This organism frequently causes invasive infections in neutropenic patients and those undergoing solid-organ or hematopoietic stem cell transplants [17]. Invasive infections caused by *S. aureus* continue to increase in frequency [18, 19]. The increase in incidence of serious infections caused by *S. aureus* is concerning given the high mortality associated with *S. aureus* bacteremia and endocarditis (15 to 40%), even with appropriate antimicrobial therapy [18, 20, 21]. In addition, over the past decade *S. aureus* has become increasingly resistant to available antimicrobials [22]. To date, there are no licensed prophylactic or therapeutic vaccines either for *S. aureus* or *Candida*.

The agglutinin-like sequence 3 protein (Als3p) of *C. albicans* is both an adhesin [23] and an invasin [24] for *Candida*. It also has both sequence and structural homology with cell surface proteins on *Staphylococcus aureus* [23]. These findings led to its evaluation as a vaccine antigen where it was demonstrated to have protective efficacy in preclinical animal models of oral, vaginal and disseminated candidiasis as well as disseminated staphylococcemia [25–27] and *S. aureus* skin and soft tissue infection (unpublished data). Additionally, this protective immunity was effective against several species of *Candida* [25] and against several clinical isolates of *S. aureus* [27]. Finally, the vaccine was shown to be highly immunogenic in animal models, inducing robust anti-Als3p B-cell response in mice [28], rabbits and non-human primates (unpublished data) along with robust T-cell responses in mice [29].

Based on these preclinical observations, purified Als3p bulk was manufactured under current Good Manufacturing Practices (cGMP), incorporated into formulations containing aluminum hydroxide as an adjuvant (designated as NDV-3 vaccine) and evaluated in this clinical study. The results of evaluating the safety, tolerability and immunogenicity of NDV-3 vaccine in healthy volunteers are presented below.

Methods

Study design

The study was a double-blind, placebo-controlled, ascending dose escalation study (30 and 300 µg) that enrolled healthy adults at a single study site. Vaccinations occurred on study day 0, with follow up evaluations on study days 3, 7, 14, 28, 90 and 180. A subset of vaccinees was re-consented to receive a second dose of vaccine on study day 180, with follow up visits 7, 14, and 90 days after the second dose. The lower participation rate in receiving the second dose (9 of 15 for the 30 µg dose and 10 of 15 for the 300 µg dose) was documented as primarily due to the timing of the second dose and follow-up conflicting with mid-summer personal schedules. Details of the study design and execution are provided in Supplemental Materials.

Vaccine and adjuvants

The active component of the NDV-3 vaccine is a recombinant version of the N-terminal region (416 amino acids) of the *C. albicans* Als3p with the addition of a six-His tag and linker sequences [26]. Als3p was produced by batch fermentation of a *Saccharomyces cerevisiae* expression cell line at 100 L scale, harvested by centrifugation and purified using two chromatography columns (nickel-affinity and hydrophobic interaction resins) followed by concentration, diafiltration into phosphate-buffered saline (PBS), pH 7, and filtration. The purified Als3p bulk drug substance was intact, monomeric and 99% pure by SDS-PAGE with Coomassie staining and was formulated with aluminum hydroxide at 1.0 mg Al/mL in PBS, pH 7. Two final container vaccine clinical lots were used for this study; lot 0939 (60 µg Als3p/mL) and lot 0940 (600 µg Als3p/mL). Clinical lots were stored at 2–8°C post manufacture and monitored for stability. Manufacture of the bulk drug substance and final container lots using cGMPs was conducted by Althea Technologies (San Diego, CA).

Immunogenicity analysis

Blood samples were obtained from subjects on the specified days post vaccination. Plasma and PBMCs were isolated using standardized procedures. Plasma samples were evaluated for anti-Als3 total IgG and for anti-Als3 IgA1 by standardized ELISA methodology. Results are expressed in units of dilution⁻¹. PBMCs were evaluated by ELISpot analysis to determine the portion of cells that could be stimulated to produce IFN-γ or IL-17A (two separate assays). Results are expressed in units of spot forming units (SFU) per 10⁶ cells. Details of the above procedures are provided in Supplemental Materials.

Statistical Analyses

Statistical analysis of assay results used non-parametric analysis using the Wilcoxon rank-sum test [30]. Evaluation of trends across groups used the Kruskal-Wallis test [31].

Results

An overview of the clinical study design (Figure S1) and execution (Table S1) are provided in Supplemental Materials.

Safety

In this study population, NDV-3 was safe and generally well-tolerated after one or two doses. Local injection site reactions to placebo (post dose 1) and vaccine and (post dose 1 and 2) are summarized in Table 1. The most common complaint was injection site pain, typically mild, lasting 1–2 days after vaccination and resolving within 2–3 days without sequelae.

The systemic and injection site adverse events (AEs) occurring in at least two study subjects after either the first or the second dose are presented in Table 1. After dose 1 each of the systemic AEs shown in Table 1 were reflected in 2–3 of the 40 subjects. After dose 2, the most common systemic AEs were fatigue and headache (5 out of 19 (26%) subjects for each). Systemic AEs were usually mild and occasionally moderate, but all resolved without sequelae within a few days. There were no notable differences in systemic AEs between the two dose levels.

Immune Response

Plasma Anti-Als3p Total IgG and IgA1—Prior to vaccination (day 0), 36 of the 40 subjects exhibited a detectable pre-existing anti-Als3p total IgG titer ranging from 114 to 2608 dilution⁻¹, with 4 subjects showing IgG titers below the limit of detection (LOD) of the assay (<50 dilution⁻¹). For IgA1 titers, 36 of the 40 subjects exhibited pre-existing detectable anti-Als3p IgA1 titer ranging from 102 to 6473 dilution⁻¹, with 4 subjects showing IgA1 titers below the LOD (<50 dilution⁻¹). Two subjects had no detectable anti-Als3p IgG or IgA1 prior to vaccination.

The geometric mean of anti-Als3p total IgG titers (Figure 1A) and IgA1 titers (Figure 1B) rose quickly after the first dose of vaccine, showing a substantial rise by day 7 post vaccination and reaching a maximum by day 14 post vaccination. The IgG and IgA1 titers from day 7 on were significantly higher for the 300 µg dose relative to the 30 µg dose (Mann-Whitney test, $p < 0.05$) and both were beyond the range of placebo recipient titers (Mann-Whitney test, $p < 0.001$). Antibody titers out to 180 days post vaccination showed roughly a two-fold decline from the maximum titers.

Following the second dose of vaccine, marked increases in anti-Als3p total IgG and IgA1 were noted, with the increase in the geomean IgG titer of the 30 µg recipients being greater than that of the 300 µg recipients.

The fold-rise of anti-Als3p total IgG titers (Figure 2A) and IgA1 titers (Figure 2B) above the pre-vaccination titers all peaked at 14 days post vaccination. The fold-rise for IgG from day 7 to 180 was significantly higher for the 300 µg dose relative to the 30 µg dose (Mann-Whitney test, $p < 0.05$) and for both dose levels the mean fold-rise remained >7-fold above pre-vaccination titers through day 180 post vaccination. Based on a definition of seroconversion being a 4-fold rise in antibody titer relative to the pre-vaccination antibody titer, a single dose of NDV-3 resulted in 13% and 53% of subjects seroconverting in IgG by day 7 (30 and 300 µg dose, respectively) and 100% seroconverting by day 14 in both dose groups (see Table 2). Similar results were observed for IgA1 seroconversion. For the 300 µg dose group, seroconversion remained at 100% for IgG through day 180, although for IgA1 there was a decline from 100% after day 28. For the 30 µg dose group, the seroconversion rate for IgG declined from 100% after day 28, while IgA1 seroconversion rates declined from 100% after day 14 (Table 2).

When study subjects were given a second dose of vaccine identical to their first, the increase in antibody titer after 14 days was relatively modest for those receiving the 300 µg dose, with increases in the GMT for IgG and IgA1 of 1.8- and 2.0-fold (data not shown). For those

receiving the 30 µg dose the GMT of IgG titers after 14 days increased 4.1-fold and the GMT for IgA1 increased 2.4-fold. At either dose level, the kinetics of the decrease in IgG and IgA1 titers appears to resume about the same rate as seen after the first dose (Figure 1).

Anti-Als3 IFN-γ and IL-17A Production by stimulated PBMCs—Figure 3 provides an overview of the IFN-γ (panel A) and IL-17A (panel B) ELISpot results for subjects receiving the 30 µg dose of vaccine. Subjects at each time point were segregated into “non-responders” and “responders”, depending on whether or not their result exceeded the empirical cutoff of 25 SFU per 10⁶ cells. For both IFN-γ and IL-17A, the maximum response was seen on day 28 post vaccination. The mean responses (SFU per 10⁶ cells) for responders in each assay were similar on days 7 to 180, with a significantly greater number of cells producing IFN-γ than IL-17A.

Figure 4 provides an overview of the ELISpot results for subjects receiving the 300 µg dose of vaccine. In this case subjects receiving 300 µg of Als3p responded more rapidly than those receiving 30 µg Als3p, i.e. by day 7, and with a greater number of cells producing both cytokines. However, the drop off of response appeared to be more rapid, with a diminished response at day 90 post vaccination for both cytokines. Again, the IFN-γ producing cells far outnumbered the IL-17A cells in the responders at all time points.

Figure 5 presents ELISpot data as the percent of positive responders for each group, including placebo recipients, at each time point. While this presentation reinforces the observations described above, it also makes clear that the IFN-γ response was more robust than the IL-17A response, with a greater percent of subjects responded at each time point and a greater difference from the placebo response. There was a more rapid rise in the percentage of both IFN-γ and IL-17A responders in the 300 µg dose group versus the 30 µg dose group on day 7 post-vaccination. However, by day 28 the 30 µg dose group showed slightly higher response rates than seen in the 300 µg dose group. Furthermore, administration of the second dose at day 180 markedly increased the percentage of responders, with the IFN-γ response elevated most by the 30 µg dose and the IL-17A response elevated most by the 300 µg dose.

Discussion

Data from this first-in-human study suggests that the NDV-3 vaccine at both 30 and 300 µg doses is generally well-tolerated. Local site reactions were fairly typical of an intramuscular vaccine formulated with an aluminum adjuvant, with no notable difference between dose levels, but with injection site pain and swelling showing a trend of increasing after dose 2. Systemic AEs at both dose levels and after both doses were generally mild and resolved quickly without sequelae. The fact that there were no serious AEs, that the mild or moderate AEs at either dose level and after both doses resolved within a few days and that there were no notable differences between the two dose levels after each dose, suggests that this protein antigen is safe in this study population (healthy adults 19–47 years old).

The immune response assays employed in this study were based on the accumulated preclinical evaluation of NDV-3 in mice that suggested that the primary B-cell responses of interest were production of total IgG and IgG2a [28] and the primary T-cell responses of interest were Als3-stimulated production of IFN-γ and IL-17A [29]. While additional Als3-stimulated immune responses could have been measured, evaluation of other responses (e.g. other antibody isotypes and Th2 responses) in mice [28] and in unvaccinated humans [32] did not present compelling evidence that these were as likely to provide future surrogate markers of protection. Furthermore, in this first study of NDV-3 in humans, sampling was confined to blood draws at defined time points to permit evaluation of the magnitude and

kinetics of these immune response markers without being overly invasive. Thus, the current results set the stage for future studies i.e. localized sample collection (e.g. cervicovaginal washes), additional isotype analysis and further cytokine profiling to identify potential immune biomarkers of greatest relevance to understanding the mechanisms of NDV-3 response and potential efficacy.

A total of 73 healthy adults have been evaluated for the background presence of naturally occurring anti-Als3p antibodies [40 in this study, 13 previously [32], plus 20 additional (unpublished data)]. Anti-Als3p antibodies (IgG and IgA1) were detected in 69 (95%) of these subjects. The fact that 100% of the vaccinees (n=30) in this trial responded with a rapid rise in anti-Als3p antibody titer supports the concept that natural colonization or prior infection provides a priming effect for this recombinant Als3p antigen, allowing a single dose of vaccine to elicit an anamnestic response in most people. This finding also suggests that those presenting as below the limit of detection (“<LOD”) based on testing a 50-fold dilution of serum might have shown a quantifiable response if a lower fold dilution of serum were evaluated.

As shown in Figures 1 and 2, a single dose of NDV-3 induced rapid and robust anti-Als3p IgG and IgA1 responses. Although antibody titer is considered either a correlate of protection or at least a surrogate marker of protection for many human vaccines [33], it is too early in the clinical development of NDV-3 to advance a hypothesis on the clinical significance of the antibody response. Even so, the kinetics of antibody titer rise and fall and seroconversion rates (Table 2) present a profile that is very attractive for addressing a healthcare-associated pathogen, where protection after one dose may be a critical application of a vaccine product. Preliminary data indicate that antibodies produced in subjects vaccinated with NDV-3 promoted a variety of functional responses, e.g. opsonophagocytic killing (OPK) of both *C. albicans* and *S. aureus*, blocking *C. albicans* adherence to, invasions of, and damage to host cells (data not shown).

Although there was a general increase in antibody titers after a second dose of NDV-3, both dose levels resulted in almost the same GMT for IgG as seen after the first dose of 300 µg (Figure 1). This response may reflect a maximum capacity for IgG response to this antigen. The option of one dose of a relatively high antigen dose level or two doses of a much lower antigen dose level could be a critical consideration not only for specific clinical indications, but also if Als3 was combined with other antigens in a combination vaccine product as the higher antigen dose level could lead to immune interference in combination antigens.

In contrast, the impact of the second dose on the GMT for IgA1 appears to be different; the post dose 2 peak titers for each dose level are similar to what was achieved after the first dose at each dose level (Figure 1). This response suggests that the ultimate dosing strategy will also depend in part on whether IgG and/or IgA are responsible for functional OPK activity.

NDV-3 also induced a substantial T-cell response, as evidenced by the post-vaccination increase in the number of PBMCs producing IFN-γ and IL-17A upon Als3-peptide stimulation. The time course of the number of cells producing cytokines (Figures 3 and 4) shows that the response peaked by day 28 post-vaccination, declined out to day 180, and then rebounded upon administration of a second dose to produce a median response that was similar to or greater than that seen after the first dose. When examined as the percentage of positive responders (Figure 5) it appears that, at least for IFN-γ, the first and second dose of NDV-3 produced a similar percentage of positive responders within weeks of dose administration. This finding suggests that memory T-cells induced by natural exposure to *Candida* or NDV-3 have a rapid recall response on re-exposure to Als3p. Of note, the 300

μg Als3p dose appeared to induce a more robust early response than the 30 μg dose, as there were a smaller percentage of positive responders after day 7 in the subjects who received the 30 μg Als3p dose. A similar trend was seen for the IL-17A response after the first dose, though the overall percentage of responders was lower than for IFN- γ . This outcome may suggest that the IL-17A response to the first NDV-3 dose is limited in human vaccinees. Analysis of the post dose 2 data suggests that 300 μg Als3p produces a more robust IL-17A response. Therefore, a second dose may be beneficial if an IL-17A response proves essential for protection in humans. It is interesting to note that recent work by Netea et al [34] suggests that infection by live, but not heat-killed, *Candida* suppresses the Th17 immune response.

The degree of T-cell stimulation by NDV-3 is remarkable for a protein antigen with an aluminum adjuvant if for no other reason than the lack of precedent for measuring human T-cell responses from a recombinant protein antigen. For example, IFN- γ producing cells reached a median level of >200 SFU/ 10^6 cells at most time points evaluated and rose to >1000 SFU/ 10^6 cells in some subjects. These levels are on the order of those seen with DNA and viral vector vaccines in humans [35, 36] and are suggestive of a robust Th1 response induced by this vaccine. In addition, a majority of subjects demonstrated an elevation of IL-17A-producing cells, suggesting NDV-3 induces a mixed Th1-Th17 immune enhancement. This same pattern was previously seen in mice, where Als3p-specific IFN- γ and IL-17-secreting T cells appear to be the protective mechanism of NDV-3 against both *Candida* and *S. aureus* [36]. Considerable recent attention has been given to IL-17 secreting CD4 $^{+}$ (Th17) cells and their potential role in vaccine-induced immunity to a diverse array of bacteria and viruses in preclinical models [37].

Animal models may not parallel the human condition of universal pre-exposure to commensal pathogen (*Candida* or *S. aureus*) prior to the onset of disease. Moreover, most humans appear to have a modest but pre-existing Als3-specific IFN- γ and IL-17A response that can be enhanced upon vaccination. While it is conceivable that an enhanced non-specific IL-17 response could be detrimental, (e.g. inducing organ-specific and systemic autoimmune diseases [38]), the lack of AEs observed in this study is a preliminary indication that this is not an issue with this vaccine in this healthy adult population. The precise role of an enhanced antigen-specific Th17 immune response in humans in combating candidal or staphylococcal disease is not known. However, it is clear that a genetic deficiency of Th17 immune pathway results in greater susceptibility of humans to infection by these and other pathogens [38]. These collective findings support continued exploration of the Th1 and Th17 responses to NDV-3 as a potentially important contributors to vaccine efficacy for *Candida* and *S. aureus* [39].

The decision to use a saline placebo for this study was based in part on preclinical studies with NDV-3 in non-human primates which showed no enhancement of Als3-specific responses in animals receiving alum-containing placebo (unpublished data) and a decision to not impose on placebo recipients the local site AEs that are routinely associated with administration of aluminum hydroxide. This is a typical design feature for Phase 1 non-viral vaccine studies conducted in the US over the past decade (see www.clintrials.gov). While done with good intent and acceptable to the FDA, use of a saline placebo does limit knowledge of the “control” response to all vaccine components in the absence of the specific antigens. When dealing with antigens from commensal organisms, for which study participants are expected to be pre-primed, as well as antigens and adjuvants that each induce inflammatory responses, there is the risk that use of a vaccine containing an adjuvant as compared to a saline control will mistake non-specific responses (due to the adjuvant) for antigen-specific responses. Based on the non-human primate study, this risk seemed minimal.

In a similar vein, no first-in-human study can cover all of the biologically relevant questions about the impact of a new vaccine candidate on human recipients. Questions about gut microbial impact, other coincident pro-inflammatory responses that were not monitored in this study and the magnitude and cell-type specific origin of the cytokine responses were simply beyond the scope of this first-in-man study and will be considered, along with the use of an adjuvant-containing placebo, in future studies.

Currently *S. aureus* vaccines appear to be a consensus target for development by large pharmaceutical companies, with Pfizer, GlaxoSmithKline, Merck, sanofi pasteur, Novartis and others showing activity in this field. The mainstream of development has focused on antibody production and opsonophagocytic function rather than on T-cell responses [40]. Results reported here provide a sound basis for further exploration of the potential importance of T-cells in the protective immune response to this bacterium, including MRSA. Future studies with the investigational NDV-3 vaccine will evaluate whether the humoral response and/or cellular and cytokine responses are more effective in prevention of staphylococcal and/or candidal infections in Phase II clinical trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights for NDV-3 manuscript

- NDV-3 is safe and generally well-tolerated in healthy adults
- NDV-3 elicits quick and robust B- and T-cell immune responses
- A single dose induces an anamnestic rather than priming immune response
- T-cell response includes increases in PBMCs producing IFN- γ and/or IL-17A
- A second dose further enhances the B- and T-cell immune response

Figure 1. Plasma Anti-Als3 Total IgG and IgA1 Titers through Day 270 post vaccination
 Plasma was collected from all subjects at several time points and analyzed for anti-Als3 total IgG and IgA1 titers using indirect ELISA (see “Methods”). Geomean anti-Als3p IgG (**panel A**) and IgA1 (**panel B**) values and standard deviations of the geomean are shown, though the latter are generally within the bounds of the symbol at each time point.

Figure 2. Fold-Rise Increases in Plasma Anti-Als3p Total IgG and IgA1 through Day 270 post vaccination

The fold-rise in antibody titer is calculated for each subject as the ratio of the antibody titer at each time point post vaccination to the pre vaccination titer (day 0). For a given group and time point, the fold-rise is calculated as the geomean of the fold-rise values for all individuals in the group for total IgG (**panel A**) and IgA1 (**panel B**). Geomean values and standard deviations of the geomean are shown for each group and time point. The dashed line represents a 4-fold rise in antibody titer.

Figure 3. Als3-stimulated IFN- γ and IL-17A production by PBMCs for all subjects receiving the 30 μ g dose through Day 270 post vaccination

PBMCs were collected from all subjects at several time points and were analyzed for Als3-specific stimulation of IFN- γ (**panel A**) or IL-17A (**panel B**) production using ELISpot assay (see “Methods”). Values less than 2 are plotted as a cloud at the bottom of the stack so that all subjects are represented on the figure. The definition of a responder in either assay is an ELISpot result that is a ≥ 25 SFU per 10^6 cells difference in Als3 peptide-stimulated cultures minus unstimulated cultures (dashed line). The median value (—) for each data set is shown.

Figure 4. Als3-stimulated IFN- γ and IL-17A production by PBMCs for all subjects receiving the 300 μ g dose through Day 270 post vaccination

PBMCs were collected from all subjects at several time points and were analyzed for Als3-specific stimulation of IFN- γ (**panel A**) or IL-17A (**panel B**) production using ELISpot assay (see “Methods”). Values less than 2 are plotted as a cloud at the bottom of the stack so that all subjects are represented on the figure. The definition of a responder in either assay is an ELISpot result that is a ≥ 25 SFU per 10^6 cells difference in Als3 peptide-stimulated cultures minus unstimulated cultures (dashed line). The median value (—) for each data set is shown.

Figure 5. Percentage of Subjects Responding with IFN- γ and IL-17A producing PBMCs through Day 270 post vaccination

The plotted results represent the percentage of individuals in a given group and time point that had a valid IFN- γ (**panel A**) or IL-17A (**panel B**) ELISpot response that exceeded the specified cutoff (25 SFU per 10^6 cells difference in Als3 peptide-stimulated cultures minus unstimulated cultures).

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Systemic and injection side AEs reported Days 0–7 post-vaccination regardless of causality. AEs included below reflect any AE that was reported by at least 2 vaccinees during that time frame, i.e. out of 40 total post-dose 1 and out of 19 post-dose 2.

Table 1

MedDRA Preferred Term	Placebo Dose 1 N=10	30 µg Dose		300 µg Dose	
		Dose 1 N=15	Dose 2 N=9*	Dose 1 N=15	Dose 2 N=10*
<u>Injection site</u>					
Erythema	10%	20%**	11%***	0	10%**
Induration	10%	0	0	0	20%**
Pain	20%	73%**	100%	73%#	100%**
Swelling	0	7%	22%***	7%	30%**
<u>Systemic AEs</u>					
Diarrhea	0	7%	11%	7%	0
Nausea	0	13%	0	0	30%
Fatigue	10%	7%	11%	7%	40%
Myalgia	0	0	11%	20%	20%
Pain in extremity	0	0	0	13%	10%**
Headache	10%**	7%	22%	7%**	30%

* Subjects volunteered to continue in study to receive a 2nd dose.

** One graded as “moderate”.

Three graded as “moderate”.

*** One graded as “severe”.

All AEs resolved without sequelae.

Table 2

Fold rise of anti-Als3 antibody titer relative to pre-vaccination (Day 0) titers.

Dose (µg)	Ig type	% of subjects with 4-fold rise in anti-Als3 antibody titer			
		Day 7	Day 14	Day 28	Day 90
30	IgG	13	100	100	93
300	IgG	53	100	100	100
30	IgA1	7	100	93	87
300	IgA1	60	100	100	87