Title

A Fungal Immunotherapeutic Vaccine (NDV-3A) for Treatment of Recurrent Vulvovaginal Candidiasis-A Phase 2 Randomized, Double-Blind, Placebo-Controlled Trial.

Journal

Clinical Infectious Diseases, 66(12)

Authors

Edwards, John
Schwartz, Michael
Schmidt, Clint
et al.

Publication Date

2018-06-01

DOI

10.1093/cid/ciy185

Peer reviewed

Clinical Infectious Diseases

MAJOR ARTICLE

A Fungal Immunotherapeutic Vaccine (NDV-3A) for Treatment of Recurrent Vulvovaginal Candidiasis—A Phase 2 Randomized, Double-Blind, Placebo-Controlled Trial

John E. Edwards Jr,[1,2] Michael M. Schwartz,[3] Clint S. Schmidt,[3] Jack D. Sobel,[4] Paul Nyirjesy,[5] Florian Schodel,[6] Erica Marchus,[3] Mary Lizakowski,[3] Elizabeth A. DeMontigny,[3] Jesse Hoeg,[3] Tuomas Holmberg,[3] M. Timothy Cooke,[3] Keila Hoover,[7] Lance Edwards,[8] Mark Jacobs,[9] Steven Sussman,[10] Michael Augenbraun,[11] Michael Drusano,[12] Michael R. Yeaman,[1,2] Ashraf S. Ibrahim,[1,2] Scott G. Filler,[1,2] and John P. Hennessey Jr[3]

[1]Los Angeles Biomedical Research Institute, Torrance, and [2]David Geffen School of Medicine at University of California, Los Angeles; [3]NovaDigm Therapeutics, Inc, Boston, Massachusetts; [4]School of Medicine, Wayne State University, Detroit, Michigan; [5]Drexel University College of Medicine, and [6]Philimmune LLC, Philadelphia, Pennsylvania; [7]Miami Clinical Research, Corp, Florida; [8]Suffolk Obstetrics & Gynecology, Port Jefferson, New York; [9]TMC Life Research, Inc., Houston, Texas; [10]Lawrence OB-GYN Clinical Research, LLC, Lawrenceville, New Jersey; [11]Research Foundation of SUNY, Albany, New York; and [12]Community Medical Research LLC, Miami Beach, Florida

(See the Editorial Commentary by Casadevall and Pirofski on pages 1937–9.)

Background. Recurrent vulvovaginal candidiasis (RVVC) is a problematic form of mucosal *Candida* infection, characterized by repeated episodes per year. *Candida albicans* is the most common cause of RVVC. Currently, there are no immunotherapeutic treatments for RVVC.

Methods. This exploratory randomized, double-blind, placebo-controlled trial evaluated an immunotherapeutic vaccine (NDV-3A) containing a recombinant *C. albicans* adhesin/invasin protein for prevention of RVVC.

Results. The study in 188 women with RVVC (n = 178 evaluable) showed that 1 intramuscular dose of NDV-3A was safe and generated rapid and robust B- and T-cell immune responses. Post hoc exploratory analyses revealed a statistically significant increase in the percentage of symptom-free patients at 12 months after vaccination (42% vaccinated vs 22% placebo; $P = .03$) and a doubling in median time to first symptomatic episode (210 days vaccinated vs 105 days placebo) for the subset of patients aged <40 years (n = 137). The analysis of evaluable patients, which combined patients aged <40 years (77%) and ≥40 years (23%), trended toward a positive impact of NDV-3A versus placebo ($P = .099$).

Conclusions. In this unprecedented study of the effectiveness of a fungal vaccine in humans, NDV-3A administered to women with RVVC was safe and highly immunogenic and reduced the frequency of symptomatic episodes of vulvovaginal candidiasis for up to 12 months in women aged <40 years. These results support further development of NDV-3A vaccine and provide guidance for meaningful clinical endpoints for immunotherapeutic management of RVVC.

Clinical Trials Registration. NCT01926028.

Keywords. *Candida*; RVVC; candidiasis; vaccine; Als3.

Currently, there are no licensed vaccines or immunotherapeutics against fungal infections. Healthcare-associated and community-acquired *Candida* infections have substantially increased in prevalence in recent years, as has antifungal resistance [1]. Globally, a substantial number of women are afflicted with vulvovaginal infections due to *Candida spp.* Most notable is recurrent vulvovaginal candidiasis (RVVC), which is estimated by self-reported diagnosis to impact 6%–9% of women in the United States [2], with an estimated 138 million women worldwide affected by RVVC annually and 492 million affected with RVVC at some point during their lifetime [2]. Although RVVC has been historically defined by some as ≥4 episodes per year, in practice women with ≥3 episodes per year are treated similarly in clinical settings [2].

Recurrent vulvovaginal candidiasis has a substantially negative impact on quality of life related to symptoms, frequency, and unpredictability [3]. Currently, RVVC is treated with repeated or prolonged antifungal therapy, which has variable efficacy, poses specific safety concerns [4], and adds selective pressure for antifungal resistance [5]. Thus, a safe and effective vaccine would represent a substantial improvement in management of RVVC. Importantly, a vaccine effective against RVVC could lead to a vaccine against life-threatening *Candida* infections, including those caused by drug-resistant isolates.

Received 30 October 2017; editorial decision 5 February 2018; accepted 1 March 2018; published online April 25, 2018.

Correspondence: J. P. Hennessey Jr, NovaDigm Therapeutics, 1622A Beacon Street, Suite 302, Brookline, MA 02446 (john_hennessey@novadigm.net).

Clinical Infectious Diseases® 2018;66(12):1928–36

DOI: 10.1093/cid/ciy185

This study explored the safety, immunogenicity, and impact on vulvovaginal candidiasis (VVC) recurrence of the NDV-3A vaccine in women with RVVC. This vaccine contains the N-terminal portion of the agglutinin-like sequence 3 (Als3) protein of *Candida albicans,* a hyphal-specific virulence factor that mediates adherence to [6] and invasion of [7] human epithelial and vascular endothelial cells. The vaccine has undergone extensive preclinical evaluation in hematogenously disseminated, oropharyngeal, and vaginal candidiasis murine models [6–18]. Additionally, it is highly immunogenic in nonhuman primates (J. P. Hennessey Jr, NovaDigm Therapeutics, unpublished data) and safe and immunogenic in phase 1 studies in humans [19].

Here we present the results of an exploratory phase 1b/2a study of NDV-3A versus placebo to evaluate the safety, immunogenicity, and impact on recurrence and time to first recurrence of VVC in women with RVVC. To our knowledge, this is the first such study of a purified recombinant fungal protein vaccine.

METHODS

Clinical Trial Materials

Vaccine and placebo lots used in this study were manufactured using current Good Manufacturing Practices, stored at 2°C–8°C and monitored for stability. The recombinant Als3 antigen in NDV-3 (Als3 fused to a 6-His tag and linker sequences) and in NDV-3A (Als3 with no extraneous sequences) were produced in *Saccharomyces cerevisiae*, purified (>98% purity), and formulated with aluminum hydroxide as described previously [19]. NDV-3 was used in the phase 1 studies, but NDV-3A is the form of the vaccine that is the focus of further development. The placebo lot contained all vaccine components with the exception of Als3.

Study Design

This was a double-blind, placebo-controlled study designed to enroll 189 patients, which was conducted from July 2013 to May 2016 in 20 study sites within the continental United States. The phase 1b portion of this study included 15 patients per group and compared the safety and immunogenicity among NDV-3, NDV-3A, and placebo groups. The phase 2a portion of the study was designed with 80% power to detect a difference in efficacy of NDV-3A and placebo, with α (2-sided) of 0.05, assuming a 50% attack rate (over 6 months) in placebo recipients, an estimated vaccine efficacy of 50%, and an expected early withdrawal rate of 25%. This design called for 87 patients each in the NDV-3A and placebo groups. The comparison provided a bridge to assure that NDV-3A safety and immunogenicity results can be linked back to those generated using NDV-3 in initial phase 1 studies.

Enrolled patients provided informed consent and met all inclusion and exclusion criteria for the study. In part, these criteria required patients be aged 18–55 years, using an approved method of birth control, and presenting with a clinically diagnosed active VVC episode at time of enrollment. An active VVC episode was defined as having a composite sign and symptom score [20] of ≥3 (range, 0–18), a vaginal swab culture-positive for *C. albicans*, and at least 2 episodes of vaginitis during the 12 months prior to the current episode, 1 of which was documented by a diagnostic test specific for *Candida*. A complete list of inclusion and exclusion criteria and demographic data can be found in Supplementary Tables S1–S3.

Enrolled patients received 3 doses of oral fluconazole (150 mg each), taken on days −14, −11, and −8. At day 0, those with sign/symptom scores ≥3 were exited from the study and treated per the judgement of their physician. Those with combined sign/symptom scores <3 and who still met inclusion/exclusion criteria were randomized to a single intramuscular dose of either vaccine or placebo and were given 3 more doses of fluconazole (150 mg each), taken on days 0, 7, and 14 to allow near maximum immune response to the vaccine with minimal chance of influence of an active *Candida* colonization.

Enrollees were scheduled for 7 office visits and allowed unlimited unscheduled visits to address questions or symptoms. At all visits enrollees underwent a complete history, including a review of adverse events and medications being taken, physical examination, symptom scores (by the patient), sign scores (by the investigator), mycological culture of vaginal swabs, and a cervicovaginal (CV) wash. Additionally, blood was collected for isolation of serum and peripheral blood mononuclear cells (PBMCs). All samples were stored at <−60°C.

Patients who presented at office visits after day 17 (the 3 half-lives wash-out period for fluconazole) with symptom scores ≥3 were considered recurrent cases of VVC regardless of the culture result of their vaginal swab, treated with oral fluconazole (150 mg per day every 3 days for 3 doses), and continued in the study. A recurrence of VVC in vaccinated patients within 17 days of a previous episode was considered a failure of fluconazole therapy and not counted as a new episode of VVC.

Immunological Assays

Serum and CV wash samples were analyzed for anti-Als3 immunoglobulin G (IgG) and immunoglobulin A1 (IgA1), and PBMCs were evaluated for Als3-specific production of interferon γ (IFN-γ) and interleukin 17A (IL-17A) as previously described [19].

Statistical Analyses

The statistical analyses of this study were consistent with the International Conference on Harmonisation (ICH) guidelines E9: Statistical Principles for Clinical Trials [21]. For each outcome, the proportion of patients in each group was summarized with point estimates and their 95% confidence interval (CI). The difference between the proportions and their 95% CIs were calculated for each outcome.

Given no prior vaccine studies in this population, this study was defined as exploratory to identify potential endpoints for subsequent studies of the efficacy of NDV-3A in women with RVVC.

RESULTS

Study Performance

Details of the screening, enrollment, and clinical visit activities are summarized in Figure 1. A total of 408 patients were screened; 65 were excluded based on criteria at study day −14. The remaining 343 patients with acute vaginitis were treated with oral fluconazole and evaluated on study day 0. Of these, 151 had a study day −14 swab that was either negative for any *Candida* species (n = 142) or positive for a non-*albicans Candida* species (n = 9), and 4 were found to have a composite score at study day 0 that was ≥3, indicating lack of resolution of VVC. These and other reasons for screen failure are provided in the Supplementary Data (Supplementary Table S1). These 151 screen failures were not continued in the study or vaccinated. The remaining 188 patients met all inclusion/exclusion criteria and were randomized and vaccinated with either NDV-3A (n = 89), placebo (n = 85), or the phase 1 vaccine candidate, NDV-3 (n = 14). The 3 patient groups had no significant differences for age, weight, body mass index, ethnicity and race (Supplementary Table S2 and S3).

During the planned 12-month postvaccination period, 45 patients (23.9%) withdrew from the study prior to completing their 12-month visit; 143 patients met all inclusion/exclusion criteria and completed the study (Table 1).

Safety

Table 2 summarizes treatment-emergent systemic adverse events (AEs) that occurred in >4% of vaccinees. Almost all AEs were mild or moderate and were not related to study treatment. Four patients in each of the NDV-3A and placebo groups reported an AE of severe intensity; all were considered unrelated to study treatment except 1 in the NDV-3A group, who had headache and muscle aches of severe intensity, not considered serious. Overall, there were no significant differences in AE rates among the NDV-3A, NDV-3, and placebo groups.

Table 3 shows that the majority of patients receiving NDV-3A or placebo experienced at least 1 injection site reaction (87.6% and 84.7%, respectively). For each specific injection site reaction there was a slightly greater frequency of AE for NDV-3A recipients than for placebo recipients, although the differences are not statistically significant.

Microbiology

The frequency of positive cultures for postvaccination (day 18 to 12 months) vaginal swabs was not significantly different (P = .13) for vaccine (NDV-3A + NDV-3; n = 299/691; 43.3%) and placebo (n = 252/643; 39.2%) recipients.

Immunogenicity

Both NDV-3A and NDV-3 elicited robust immunologic responses, with serum anti-Als3 IgG and IgA1 titers significantly higher in the vaccine groups than the placebo group at all time points beyond day 0 (Table 4). In all groups, geomean serum antibody titer reached a maximum value on day 14, declining by 4- to 8-fold after 12 months. The CV wash antibody titer maxima occurred on day 14–28, with similar levels of decline out to 12 months as seen in sera (Table 5). Differences in antibody titers between NDV-3 and NDV-3A were not statistically significant.

Stimulation of Als3-specific production of IFN-γ and IL-17A was equivalent in NDV-3A and NDV-3 recipients, with both groups having greater responses than the placebo group at day 14. Vaccinees showed a slight reduction in geomean levels of these cytokines by day 90 (Table 6).

Scheduled Versus Unscheduled Office Visits

Post hoc analysis of the exploratory outcomes measures in this study showed that patient symptom scores (range, 0–9) on

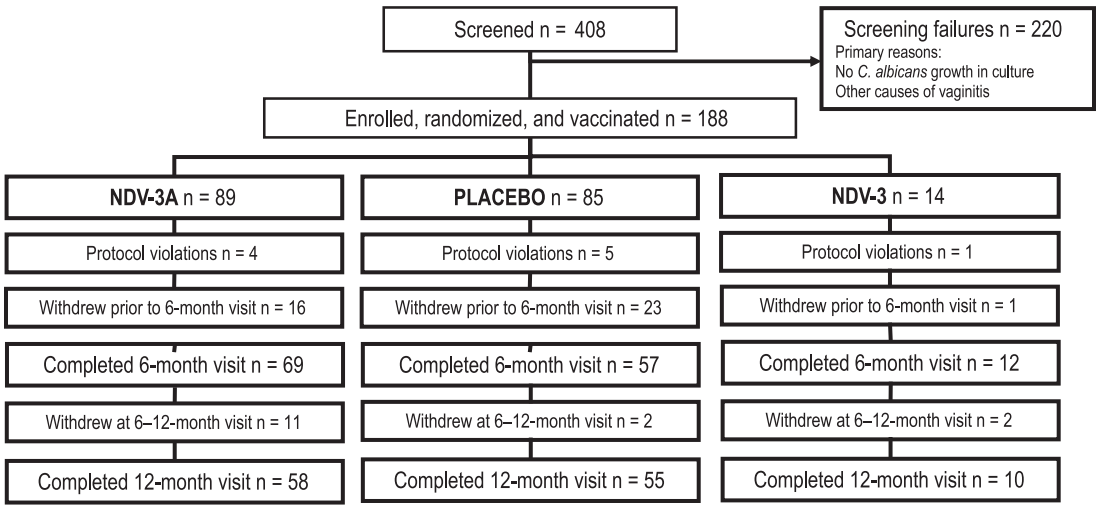

Figure 1. Flow chart for patient participation in study NDV3A-006. Abbreviation: *C. albicans, Candida albicans.*

Table 1. Summary of Patient Disposition for Those Who Were Vaccinated (n = 188)

Parameter/Category	Placebo	NDV-3A Vaccine	NDV-3 Vaccine	All Patients
Subjects vaccinated	85	89	14	188
Subjects completing the study	64 (75.3%)	67 (75.3%)	12 (85.7%)	143 (76.1%)
Subjects discontinued from study prior to 12-month visit	21 (24.7%)	22 (24.7%)	2 (14.3%)	45 (23.9%)
Reason for discontinuation				
AE/SAE	0	0	0	0
Lost to follow-up	7 (8.2%)	6 (6.7%)	0	13 (6.9%)
Investigator decision[a]	1 (1.2%)	5 (5.6%)	0	6 (3.2%)
Withdrawal of consent after dose	9 (10.6%)	9 (10.1%)	2 (14.3%)	20 (10.6%)
Noncompliance	2 (2.4%)	0	0	2 (1.1%)
Other reasons for leaving the study prior to 12-month visit	2 (2.4%)	2 (2.2%)	0	4 (2.1%)

Abbeviations: AE, adverse event; SAE, severe adverse event.

[a]Patients that discontinued the study due to investigator decision exited the study after their first recurrence to pursue other therapies that excluded their further participation in this study (eg, maintenance fluconazole therapy).

postvaccination visits with a value >1 were seen for only 11% of scheduled visits as compared with 86% of unscheduled visits (Figure 2A). Even a score of 2 shows substantial difference in the frequency for scheduled versus unscheduled visits (4% vs 9%). Physician sign scores (range, 0–9) showed no significant correlation with patient symptom scores (Figure 2B), with wide variability in relation to any given symptom score.

Impact of NDV-3A on Proportion of Recurrence-Free Patients

Table 7 shows the number and percentage of patients who were free of recurrence of symptoms consistent with VVC, defined as a patient symptom score <3, after study day 17 and at the specified office visits. A post hoc analysis of age dependence on recurrence rates in the treatment versus placebo groups showed that vaccine efficacy appeared to decrease with increasing age, with consistent results seen for age cutoffs of 38 to 42 years. For this reason, a subset of patients aged <40 years was analyzed alongside the dataset of all patients. In the time interval from day 17 up to the 3-, 6-, and 12-month office visits, the percentage of patients who were recurrence-free was greater in the NDV-3A group than in the placebo group for those aged <40 years but was not statistically significantly different for all ages. The proportion of women who were recurrence-free at 12 months (42% vaccine vs 22% placebo; odds ratio, 0.39; 95% CI, .17–.91) is notable.

Table 2. Incidence of Treatment-Emergent Adverse Events ≥4% for any Preferred Term in Any Treatment Group and Relative Risk by System Organ Class and Preferred Term for the Per Protocol Population

System Organ Class Disorder	Placebo (n = 85)	NDV-3A (n = 89)	NDV-3 (n = 14)	Overall (n = 188)	Relative Risk (NDV-3A + NDV-3) vs Placebo
TEAE MedDRA Preferred Term	N (%)	N (%)	N (%)	N (%)	
Patients reporting at least 1 TEAE	49 (57.6%)	49 (55.1%)	6 (42.9%)	104 (55.3%)	0.96
Blood and lymphatic system disorders	5 (5.9%)	1 (1.1%)	0	6 (3.2%)	0.19
Anemia	4 (4.7%)	0	0	4 (2.1%)	0.00
Infections and infestations	38 (44.7%)	29 (32.6%)	5 (35.7%)	72 (38.3%)	0.73
Bacterial vaginitis	15 (17.6%)	13 (14.6%)	1 (7.1%)	29 (15.4%)	0.83
Urinary tract infection	16 (18.8%)	7 (7.9%)	1 (7.1%)	24 (12.8%)	0.42
Nasopharyngitis	6 (7.1%)	6 (6.7%)	1 (7.1%)	13 (6.9%)	0.96
Upper respiratory tract infection	3 (3.5%)	4 (4.5%)	0	7 (3.7%)	1.27
Lower respiratory tract infection	0	0	1 (7.1%)	1 (0.5%)	NA
Sinusitis	2 (2.4%)	3 (3.4%)	11 (7.1%)	6 (3.2%)	1.43
Nervous system disorders	5 (5.9%)	7 (7.9%)	0	12 (6.4%)	1.34
Headache	4 (4.7%)	5 (5.6%)	0	9 (4.8%)	1.19
Pregnancy, puerperium, and perinatal conditions	5 (5.9%)	7 (7.9%)	0	12 (6.4%)	1.34
Pregnancy	0	1 (1.1%)	2 (14.3%)	3 (1.6%)	NA
Abortion spontaneous	0	0	1 (7.1%)	1 (0.5%)	NA
Reproductive system and breast disorders	5 (5.9%)	7 (7.9%)	0	12 (6.4%)	1.34
Vulvovaginal discomfort	1 (1.2%)	2 (2.2%)	1 (7.1%)	4 (2.1%)	1.91

A patient is counted once if the patient reported ≥1 events in each system organ class/preferred term. Referred terms within each system organ class are sorted by decreasing total frequency.

Abbreviations: NA, not applicable; TEAE, treatment-emergent adverse event.

Table 3. Overall Summary of Injection Site Reactions for the Safety Population

Injection Site Reaction	Placebo (n = 85)	NDV-3A Vaccine (n = 89)	NDV-3 Vaccine (n = 14)	All Patients (n = 188)
Patients with at least 1 injection site reaction	72 (84.7%)	78 (87.6%)	10 (71.4%)	160 (85.1%)
Induration	25 (29.4%)	46 (51.7%)	0	71 (37.8%)
Pain	56 (65.9%)	70 (78.7%)	7 (50.0%)	133 (70.7%)
Redness	35 (41.2%)	45 (50.6%)	2 (14.3%)	82 (43.6%)
Swelling	28 (32.9%)	47 (52.8%)	2 (14.3%)	77 (41.0%)
Tenderness	64 (75.3%)	71 (79.8%)	10 (71.4%)	145 (77.1%)

A patient is counted once if the patient reported ≥1 events in each injection site reaction.

Impact of NDV-3A on Time to First Symptomatic Vulvovaginal Candidiasis Recurrence

Figure 3 shows a Kaplan-Meier survival plot and a bar chart of median time to first recurrence, reflecting when patients in the indicated groups were diagnosed with their first recurrence of VVC postvaccination. A Kaplan-Meier survival plot (Figure 3A) showed a significantly faster time-dependent decline in the percentage of recurrence-free patients aged <40 years in the placebo group relative to the NDV-3A group ($P = .03$). This finding is also reflected in Figure 3B, where median time to first recurrence for patients aged <40 years was 210 days for vaccine recipients versus 105 days for placebo recipients. This impact on time to first recurrence was statistically significant by the Cox proportional hazards model (hazard ratio, 0.59). Similar evaluations of patients of all ages had a smaller difference between NDV-3A and placebo, which was not statistically significant in either the Kaplan-Meier survival plot (Supplementary Figure S1; $P = .099$) or by the the Cox proportional hazards model (Suppelementary Figure 3B; hazard ratio, 0.71). The results were similar when the median time to first recurrence was calculated only for patients who had a recurrence (ie, eliminating recurrence-free patients from the analysis; data not shown).

DISCUSSION

This study represents our ongoing efforts to develop novel vaccines to prevent or ameliorate *Candida* infections, including both mucocutaneous and invasive candidiasis. We initially focused on women with RVVC because a large percentage of women who have this condition are generally healthy with well-functioning immune systems, as opposed to those with invasive candidiasis. That said, we were mindful that vaginal irritation, even recurrent, can have many causes. Thus the initial requirement for a vaginal culture positive for *Candida* as a requirement for entry into the study substantially increased the likelihood that that our patient complaints were associated with *Candida* infections.

As shown in Tables 1–3, NDV-3A is generally safe, as was its precursor, the 6-His-tagged NDV-3 [19], showing AEs comparable with those seen in placebo recipients. Although differences in specific injection site reactions do not reach the level of statistical significance and typically resolved within a few days, NDV-3A recipients showed a slightly higher frequency of AEs than placebo recipients. This suggests a slightly elevated reactogenicity of NDV-3A in this patient population.

Immunogenicity was equivalent between NDV-3A and NDV-3; a rapid immunologic response was seen in virtually all recipients, with peak serum antibody (IgG and IgA1) titers within 14 days of vaccination (Table 4), peak vaginal antibody titers within 14–28 days (Table 5), and peak Als3-specific production of IFN-γ and IL-17A from PBMCs in response to either vaccine by 14 days after vaccination (Table 6). Overall, NDV-3 and NDV-3A have effectively similar safety and immunogenicity profiles.

Immune responses to Als3 are similar to the anamnestic responses seen with multidose vaccines and presumably reflect

Table 4. Serum Anti-Als3 Total Immunoglobulin G Antibody Titers (0–360 Days) for the Per Protocol Population

	GeoMean Dilution^{-1} (GeoSD)					
Treatment Group	Day 0	Day 14	Day 28	Day 90	Day 180	Day 360
Total anti-Als3 IgG						
Placebo	465 (3.3)	464 (3.6)	419 (3.3)	447 (3.2)	523 (3.5)	371 (3.6)
NDV-3A	434 (3.5)	37,381 (3.9)*	31,459 (3.6)*	17,818 (3.5)*	10,852 (3.5)*	5349 (3.2)*
NDV-3	305 (3.8)	18,078 (4.6)*	13,674 (4.6)*	10,868 (3.8)*	6,297 (3.9)*	4999 (3.9)*
Total anti-Als3 IgA1						
Placebo	937 (5.6)	954 (6.4)	850 (5.5)	1062 (6.0)	982 (7.3)*	563 (7.3)
NDV-3A	1011 (5.5)	63,834 (5.1)*	43,557 (5.2)*	23,053 (5.1)*	18,215 (5.9)*	8727 (5.8)*
NDV-3	657 (3.1)	44,866 (3.7)*	30,118 (3.2)*	20,349 (3.1)*	14,633 (3.4)*	12,357 (3.1)*

P values to compare the log-transformed values of NDV-3A with NDV-3 were all >.10. *$P < .001$ for differences in the log-transformed values for vaccine versus placebo.

Abbreviations: IgA1, immunoglobulin A1; IgG, immunoglobulin G.

Table 5. Cervicovaginal Wash Anti-Als3 Antibody Titers (Day 0–360) for the Per Protocol Population

	GeoMean Dilution^{-1} (GeoSD)					
Treatment Group	Day 0	Day 14	Day 28	Day 90	Day 180	Day 360
Total anti-Als3 IgG						
Placebo	2.03 (5.27)	1.93 (5.15)	1.44 (3.63)	1.40 (3.97)	1.66 (4.55)	1.44 (5.14)
NDV-3A	1.93 (4.19)	20.67 (6.28)*	24.98 (6.97)*	14.28 (6.16)*	7.68 (6.73)*	4.36 (6.39)*
NDV-3	1.47 (4.69)	8.01 (8.09)*	9.88 (14.49)*	5.28 (4.11)*	2.72 (6.42)	2.76 (6.12)
Total anti-Als3 IgA1						
Placebo	4.86 (6.37)	4.99 (7.89)	3.44 (4.51)	2.87 (4.89)	3.13 (5.60)	3.39 (5.05)
NDV-3A	5.16 (5.38)	53.65 (7.00)*	45.96 (8.56)*	22.52 (7.50)*	17.74 (5.76)*	9.32 (7.06)*
NDV-3	2.02 (6.81)	28.24 (6.86)*	22.22 (9.61)*	5.75 (6.00)	10.17 (4.83)*	5.02 (7.41)

*$P < .001$ for differences in the log-transformed values for vaccine versus placebo.

Abbreviations: IgA1, immunoglobulin A1; IgG, immunoglobulin G.

priming of the immune system to Als3 by wild-type exposure to *Candida*, which is not unexpected, given the commensal nature of this organism. The important immunological outcomes of this study are that (1) women with RVVC had low baseline anti-Als3 titers and enzyme-linked immunospot assay (ELISPOT) results, indicating that repeated vaginal exposures to *C. albicans* does not stimulate a substantive systemic immune response, and (2) that women with RVVC responded robustly to NDV-3A, illustrating their capability of generating an anti-*Candida* immune response and no evidence of a *Candida*-specific immune defect. Immunological responses will be further presented and discussed in a subsequent article.

Because this was the first clinical study evaluating the impact of a vaccine on RVVC, it was designated as an exploratory phase 1b/2a trial [22]. The primary outcomes measure of the study was safety in the target population. Secondary outcomes measures for NDV-3A versus placebo groups were Als3-specific signals of B-cell (eg, antibody) and T-cell (eg, production of IFN–γ and IL-17A) immune responses and recurrence of VVC during a 12-month postvaccination period. The study design was a modification of a well-known study for maintenance treatment of RVVC, with weekly doses of fluconazole for 6 months [20]. Although a US Food and Drug Administration (FDA) draft guidance for studies of the antimicrobial treatment of RVVC is under development [23], there is no guidance for a vaccine or immunotherapeutic strategy to manage RVVC.

Of the exploratory findings assessed in this study, patient symptom scores were particularly informative. A threshold

Table 6. Als3-Specific Cytokine Secreting Peripheral Blood Mononuclear Cells (Day 0–90) for the Per Protocol Population

	GeoMean SFU (GeoSD)		
Treatment Group	Day 0	Day 14	Day 90
Als3-specific IFN-γ secreting PBMCs			
Placebo	2.7 (3.0)	3.0 (3.2)	3.2 (3.5)
NDV-3A	3.6 (3.3)	22.8 (5.3)*	16.6 (4.6)*
NDV-3	2.6 (3.4)	22.1 (7.7)*	15.1 (4.9)*
Als3-specific IL-17A secreting PBMCs			
Placebo	3.4 (4.1)	2.6 (3.4)	2.9 (3.5)
NDV-3A	4.2 (4.4)	9.6 (5.3)*	6.6 (3.9)*
NDV-3	5.2 (4.1)	11.1 (5.4)*	3.3 (3.7)

P values to compare the log-transformed values of NDV-3A with NDV-3 were all >.30.

*$P < .001$ for differences in the log-transformed values for vaccine versus placebo.

Abbreviations: IFN- γ, interferon γ; IL-17A, interleukin 17A; PBMC, peripheral blood mononuclear cell; SFU, spot forming units per 10^6 cells.

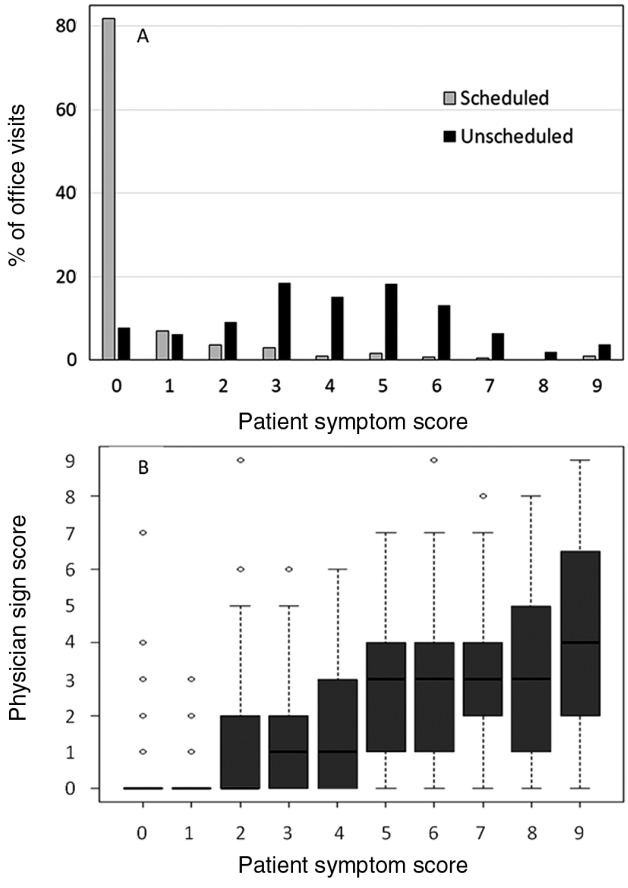

Figure 2. Evaluation of patient and physician scores. *A*, Percentage of patient office visits at each patient symptom score for scheduled and unscheduled visits. *B*, Physician sign scores versus patient symptom scores.

Table 7. Number and Percentage of Patients Who Were Recurrence-Free After Day 17, Defined by Patient-Reported Symptom Score < 3, at Selected Scheduled Visits

	Per Protocol Population Aged 18–55 Y		Per Protocol Population Aged <40 Y	
Timepoint / Endpoint	NDV-3A	Placebo	NDV-3A	Placebo
Day 17 to 3-Month Site Visit				
No. (%)	56/82 (68%)	43/77 (56%)	46/63 (73%)	31/59 (53%)
Odds ratio (95% CI)	0.59 (.31–1.12)		0.41 (.19–.87)	
P value	.11		.02	
Day 17 to 6-Month Site Visit				
No. (%)	41/80 (51%)	31/73 (42%)	35/61 (57%)	22/55 (40%)
Odds ratio (95% CI)	0.70 (.37–1.33)		0.50 (.24–1.04)	
P value	.28		.06	
Day 17 to 12-Month Site Visit				
No. (%)	28/74 (38%)	17/68 (25%)	24/57 (42%)	11/50 (22%)
Odds ratio (95% CI)	0.55 (.27–1.13)		0.39 (.17–.91)	
P value	.10		.03	

Numerator includes recurrence-free patients remaining in the study up through the specified visit. Denominator includes all patients in the study that were either recurrence-free through the specified visit or that had ≥1 recurrences at any time while in the study.

Abbreviation: CI, confidence interval.

self-assessed symptom score of ≥3 was associated with patients seeking an unscheduled clinic visit (Figure 2A). Although there was a greater percentage of unscheduled vs scheduled visits with a score of 2, this difference may be too low of a bar for definition of a recurrence. There was a wide range and variation in physician sign scores for any given patient symptom score (Figure 2B), particularly from site to site but also between physicians at a given site (data not shown). Additionally, physician sign scores did not show a relationship between the magnitude of the score and the occurance of unscheduled clinical visits.

After the initial treatment of patients with fluconazole, the culture positivity rate was relatively high (10%; data not shown) in patients with symptom scores <3. Because the vaccine was not expected to eradicate the organism (unlike in the fluconazole maintenance study), we chose to define recurrence as a return of symptoms that could be attributed to VVC. The lack of impact of the vaccine on the culture-positive status of patients may be attributed to the anti-Als3 immune response impacting the hyphal form of *Candida*, which express high levels of Als3, rather than the yeast form of *Candida*, which expresses little to no Als3 [24]. Traditional culture methods cannot discriminate between yeast and hyphal forms of *Candida*, so either form can produce a positive culture.

Because host responses to vaccines are often age-dependent, we performed post hoc analyses based on patient age and noted that the impact of NDV-3A on recurrence rates tended to be higher in patients aged <40 years. Patients aged <40 years make up 90% of those experiencing their first episode of RVVC and represented 77% of the patients in this study. These subset analyses support that NDV-3A achieves a statistically significant impact in reducing recurrence of VVC and in increasing time to first recurrence in women aged <40 years.

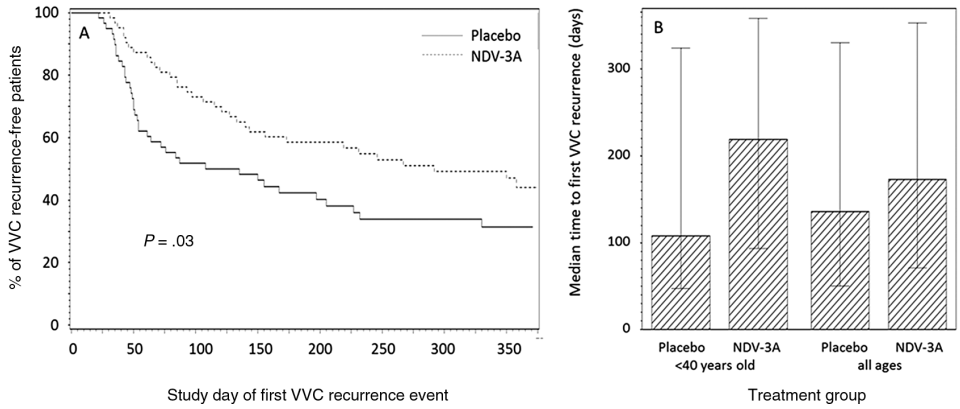

Figure 3. *A*, Kaplan-Meier survival plot of percentage of vulvovaginal candidiasis (VVC) recurrence-free patients, with VVC recurrence defined by a patient-reported symptom score ≥3, versus time after day 17, for per protocol population aged <40 years. For NDV-3A, n = 65; for placebo, n = 62. *B*, Median time to first VVC recurrence, defined by patient-reported symptom score ≥3, for each age group. Error bars reflect 1st and 3rd quartile values.

Inclusion of women aged ≥40 years in the data analysis reduced the overall impact of the vaccine, suggesting that addressing RVVC in women aged >40 years may require separate studies and perhaps a different course of treatment. The reasons that older RVVC patients may not respond as well to immunotherapy with NDV-3A remain unknown. There were no observable differences in the immune responses in RVVC patients aged ≥40 years versus younger patients. Based on a 127-patient self-reporting survey (NovaDigm Therapeutics, unpublished data), the average duration of RVVC was 3.5 years, whereas 20% of patients aged <40 years reported having had RVVC for ≥6 years versus 53% of those aged ≥40 years. Older patients who still have RVVC may have a different underlying host immune status that does not allow them to resolve the condition as readily and may also not be as amenable to the resolution of symptoms by NDV-3A immunotherapy. The impact of perimenopause and associated hormonal changes on NDV-3A efficiacy may be another area for future study.

It is also noteworthy that this study focuses on the use of NDV-3A as a therapeutic vaccine for women who are not only colonized with *C. albicans* but are having recurrent symptoms of disease. In this respect, NDV-3A is similar to shingles vaccines given to adults in that the vaccine mitigates disease from a pathogen that was present prior to vaccination.

There are several limitations of this study. First, because there is no FDA guidance for evaluation of a vaccine or immunotherapeutic agent for RVVC, we chose efficacy endpoints on a post hoc basis, which will need to be confirmed in further studies. Second, the wide range of physician scoring of clinical signs indicates a lack of standardization of sign-scoring technique. Physician scoring of signs of mucosal infection has been known to be problematic in similar studies [25, 26], and patients' symptoms may influence physician judgement regarding scoring of signs or severity. In the future, more precise definition of the signs evaluated in this study, added to a more extensive list of signs, may reduce these variations. Third, given the apparent protection of a subset of patients, despite a lack of detectable reduction in *C. albicans* burden in these patients, there may be unique challenges of vaccination against a commensual opportunistic microbe, which raise questions of whether complete decolonization is possible or desirable. Attaining culture negativity with a vaccine strategy may not be necessary, particularly if vaccines such as NDV-3A beneficially modulate host response and either modify *Candid.*burden or interfere with *Candida* transition to a pathogenic form. Finally, because this study did not include a quality-of-life assessment, some of the potential clinical measures of efficacy were not obtained. These issues will be important considerations in the design of a phase 2b trial to establish reliable clinical endpoints for further evaluation of NDV-3A in women with RVVC.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. We thank all of our clinical investigators for their contributions to this study (see [22]) and to the patients that enrolled in the study. Additionally, we thank Terrence Cochrane for managing the production and testing of the vaccine lots and Yue Fu, Don Sheppard, Brad Spellberg, Dennis Dixon, and Rory Duncan for their contributions to the preclinical development of the vaccine.

Financial support. This work was supported in part by National Institute of Health grants to J. P. H. (2R42 AI071554-02A1) and J. E. E. (R01 AI19990 and R01 AI063382) and a Department of Defense grant to J. P. H. (W81XWH-11-1-0686).

Potential conflicts of interest. J. P. H., M. T. C., T. H., C. S. S., M. M. S., E. D., M. L., E. D., and J. H. are employees and shareholders of NovaDigm Therapeutics. J. E. E., A. S. I., S. G. F., and M. R. Y. are founders and shareholders of NovaDigm Therapeutics. F. S., J. D. S., and P. N. are consultants to NovaDigm. All other authors report no potential conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: human fungal infections. Sci Transl Med **2012**; 4:165rv13.
2. Sobel JD. Recurrent vulvovaginal candidiasis. Am J Obstet Gynecol **2016**; 214:15–21.
3. Aballéa S, Guelfucci F, Wagner J, et al. Subjective health status and health-related quality of life among women with recurrent vulvovaginal candidosis (RVVC) in Europe and the USA. Health Qual Life Outcomes **2013**; 11:169.
4. Howley MM, Carter TC, Browne ML, Romitti PA, Cunniff CM, Druschel CM. Fluconazole use and birth defects in the National Birth Defects Prevention Study. Am J Obstet Gynecol **2016**; 214:657 e1–9.
5. Marchaim D, Lemanek L, Bheemreddy S, Kaye KS, Sobel JD. Fluconazole-resistant *Candida albicans* vulvovaginitis. Obstet Gynecol **2012**; 120:1407–14.
6. Sheppard DC, Yeaman MR, Welch WH, et al. Functional and structural diversity in the Als protein family of *Candida albicans*. J Biol Chem **2004**; 279:30480–9.
7. Phan QT, Myers CL, Fu Y, et al. Als3 is a *Candida albicans* invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biol **2007**; 5:e64.
8. Fu Y, Ibrahim AS, Sheppard DC, et al. *Candida albicans* Als1p: an adhesin that is a downstream effector of the EFG1 filamentation pathway. Mol Microbiol **2002**; 44:61–72.
9. Fu Y, Luo G, Spellberg BJ, Edwards JE Jr, Ibrahim AS. Gene overexpression/suppression analysis of candidate virulence factors of *Candida albicans*. Eukaryot Cell **2008**; 7:483–92.
10. Ibrahim AS, Luo G, Gebremariam T, et al. NDV-3 protects mice from vulvovaginal candidiasis through T- and B-cell immune response. Vaccine **2013**; 31:5549–56.
11. Ibrahim AS, Spellberg BJ, Avanesian V, Fu Y, Edwards JE Jr. The anti-*Candida* vaccine based on the recombinant N-terminal domain of Als1p is broadly active against disseminated candidiasis. Infect Immun **2006**; 74:3039–41.
12. Ibrahim AS, Spellberg BJ, Avenissian V, Fu Y, Filler SG, Edwards JE Jr. Vaccination with recombinant N-terminal domain of Als1p improves survival during murine disseminated candidiasis by enhancing cell-mediated, not humoral, immunity. Infect Immun **2005**; 73:999–1005.
13. Lin L, Ibrahim AS, Xu X, et al. Th1-Th17 cells mediate protective adaptive immunity against *Staphylococcus aureus* and *Candida albicans* infection in mice. PLoS Pathog **2009**; 5:e1000703.
14. Loza L, Fu Y, Ibrahim AS, Sheppard DC, Filler SG, Edwards JE Jr. Functional analysis of the *Candida albicans* ALS1 gene product. Yeast **2004**; 21:473–82.
15. Spellberg B, Johnston D, Phan QT, et al. Parenchymal organ, and not splenic, immunity correlates with host survival during disseminated candidiasis. Infect Immun **2003**; 71:5756–64.
16. Spellberg BJ, Ibrahim AS, Avanesian V, et al. Efficacy of the anti-*Candida* rAls3p-N or rAls1p-N vaccines against disseminated and mucosal candidiasis. J Infect Dis **2006**; 194:256–60.
17. Spellberg BJ, Ibrahim AS, Avenissian V, et al. The anti-*Candida albicans* vaccine composed of the recombinant N terminus of Als1p reduces fungal burden and improves survival in both immunocompetent and immunocompromised mice. Infect Immun **2005**; 73:6191–3.

18. Kamai Y, Kubota M, Kamai Y, Hosokawa T, Fukuoka T, Filler SG. Contribution of *Candida albicans* ALS1 to the pathogenesis of experimental oropharyngeal candidiasis. Infect Immun **2002**; 70:5256–8.
19. Schmidt CS, White CJ, Ibrahim AS, et al. NDV-3, a recombinant alum-adjuvanted vaccine for *Candida* and *Staphylococcus aureus*, is safe and immunogenic in healthy adults. Vaccine **2012**; 30:7594–600.
20. Sobel JD, Wiesenfeld HC, Martens M, et al. Maintenance fluconazole therapy for recurrent vulvovaginal candidiasis. N Engl J Med **2004**; 351:876–83.
21. European Medicines Agency. Use CfMPfH. ICH Topic E9: statistical principles for clinical trials: CPMP/ICH/363. **1998**. Available at: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002928.pdf.
22. Hennessey Jr, JP. Safety, tolerability, immunogenicity and efficacy of NDV-3A vaccine in preventing recurrent vulvovaginal candidiasis. Available at: https://www.clinicaltrials.gov/ct2/show/NCT01926028?term=NovaDigm&rank=1.
23. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research. Vulvovaginal candidiasis: developing drugs for treatment guidance for industry. **2016**. Available at:https://www.fda.gov/downloads/Drugs/Guidances/UCM509411.pdf.
24. Coleman DA, Oh SH, Manfra-Maretta SL, Hoyer LL. A monoclonal antibody specific for *Candida albicans* Als4 demonstrates overlapping localization of Als family proteins on the fungal cell surface and highlights differences between Als localization in vitro and in vivo. FEMS Immunol Med Microbiol **2012**; 64:321–33.
25. Lindgren C, Neuman MI, Monuteaux MC, Mandl KD, Fine AM. Patient and parent-reported signs and symptoms for group A Streptococcal pharyngitis. Pediatrics **2016**; 138. doi: 10.1542/peds.2016-0317.
26. Centor RM, Witherspoon JM, Dalton HP, Brody CE, Link K. The diagnosis of strep throat in adults in the emergency room. Med Decis Making **1981**; 1:239–46.