Immunogenicity and Smoking-Cessation Outcomes for a Novel Nicotine Immunotherapeutic

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NicVAX, a nicotine vaccine (3′AmNic-rEPA), has been clinically evaluated to determine whether higher antibody (Ab) concentrations are associated with higher smoking abstinence rates and whether dosages and frequency of administration are associated with increased Ab response. This randomized, double-blinded, placebo-controlled multicenter clinical trial (*N* = 301 smokers) tested the results of 200- and 400-µg doses administered four or five times over a period of 6 months, as compared with placebo. 3′AmNic-rEPA recipients with the highest serum antinicotine Ab response (top 30% by area under the curve (AUC)) were significantly more likely than the placebo recipients (24.6% vs. 12.0%, *P* = 0.024, odds ratio (OR) = 2.69, 95% confidence interval (CI), 1.14–6.37) to attain 8 weeks of continuous abstinence from weeks 19 through 26. The five-injection, 400-µg dose regimen elicited the greatest Ab response and resulted in significantly higher abstinence rates than placebo. This study demonstrates, as proof of concept, that 3′AmNic-rEPA elicits Abs to nicotine and is associated with higher continuous abstinence rates (CAR). Its further development as a treatment for nicotine dependence is therefore justified.

Worldwide, ~1.2 billion individuals are smokers, and ~5 million individuals die each year of illnesses caused by smoking.[1] The global rate of smoking and smoking-related deaths is anticipated to increase over the next 20 years unless significant public health measures are instituted. These include effective cessation interventions such as pharmacological treatments that improve cessation rates by 1.5- to 3-fold relative to placebo intervention.[2,3] Approved pharmacotherapies (e.g., nicotine replacements, bupropion SR, and varenicline) for smoking cessation act on the central nervous system, each with a different mechanism of action. Other novel medications are being developed, including immunotherapeutics that target nicotine.

Nicotine conjugate vaccines stimulate the immune system to develop nicotine-specific antibodies (Abs) using an immunogen comprised of nicotine covalently linked to a larger carrier protein. Conceptually, the mechanism of action is antinicotine Abs binding to nicotine molecules, and the resulting complex is too large to cross the blood–brain barrier. With increasing Ab levels, more nicotine is captured and sequestered in the blood and prevented from entering the brain, leading to a lowering of the reinforcing effects of nicotine. Studies in rats have demonstrated that passive or active immunization results in ~30–90% less nicotine entering the brain as compared with control rats,[4–7] leading to attenuation of locomotor[4,5] and behavioral[8,9] responses to nicotine. Another study in rats showed that vaccination reduces nicotine elimination from the body;[10,11] this may also contribute to reduction in smoking.

Although there are few studies in humans, published data evaluating different nicotine vaccines support the general concept that these can be effective for bringing about smoking cessation in some smokers.[12,13] Unfortunately, these studies had small sample sizes,[12] or did not use an intent-to-treat (ITT) population of smokers,[13] or did not perform statistical analysis of the data.[14]

The primary aim of this study was to establish, as proof of concept, that (i) antinicotine Abs are useful as an aid to smoking cessation and (ii) higher serum antinicotine Ab concentrations are associated with higher abstinence rates in an ITT population of smokers. One of the challenges inherent in using immunotherapeutics such as vaccines is that of achieving therapeutic levels of Abs in most people. Therefore, this study tested two

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different doses of 3′-aminomethylnicotine *Pseudomonas aeruginosa* r-exoprotein A—NicVAX (3′AmNic-rEPA)—in order to identify a dose and schedule for further development. We administered 200 and 400 μg in two separate schedules (four or five injections) and compared the effects to those of placebo with respect to immunogenicity, efficacy, and safety.

RESULTS

A total of 301 subjects were randomized to six groups (200 μg: 400 μg: placebo; two schedules for each group). **Figure 1** shows the disposition and number of subjects within each treatment group. No significant differences were observed among the groups with respect to the demographic or smoking history by treatment or Ab level (see **Table 1**).

Compliance

All 301 subjects received injection 1, and 96.7, 84.1, 72.4, and 61.2% received injections 2, 3, 4, and 5 (the latter for schedule 2 subjects only), respectively. No significant differences were observed across treatment groups in subjects receiving injections 2 through 4 for schedules 1 and 2. The mean in-study duration was 286 ± 121 days.

Proof of concept

Effects of high Ab on abstinence. High-Ab responders to 3′AmNic-rEPA were defined as the top 30% of responders by area under the curve (AUC) (0–26 weeks) and the low-Ab group as the bottom 70% of responders. 3′AmNic-rEPA recipients in the high-Ab group were significantly more likely to attain 8 weeks of continuous abstinence from smoking from weeks 19 through 26 than were those receiving placebo (24.6% vs. 12.0%, P = 0.024, odds ratio (OR) = 2.69, 95% confidence interval (CI), 1.14–6.37). No significant differences in results were observed between the 3′AmNic-rEPA low-Ab group and the placebo group (9.3% vs. 12.0%, P = 0.46). As a secondary outcome, continuous abstinence rates (CAR) to 52 weeks were evaluated from weeks 19–52; these were significantly higher in the high-Ab group relative to the placebo group (19.7% vs. 10.0%, P = 0.044, OR = 2.64, 95% CI 1.03–6.79); in contrast, there was no significant difference in results between the low-Ab group and the placebo group (7.1% vs. 10.0%, P = 0.43). The 7-day point prevalence abstinence results show that subjects with high levels of Ab were significantly more likely to abstain from smoking as compared with those receiving placebo, both at 26 weeks (36.1% vs. 16.0%, P = 0.0024, OR = 3.30, 95% CI 1.53–7.13) and at 52 weeks (31.1% vs. 12.0%, P = 0.0021, OR = 3.69, 95% CI 1.61–8.47). No significant differences were observed in the point prevalence abstinence rates between the low-Ab group and the placebo group at 26 and 52 weeks (12.9% vs. 16.0%, P = 0.51; and 11.4% vs. 12.0%, P = 0.89, respectively). As shown in **Figure 2a**, abstinence rates remained essentially the same following the target quit date (TQD) for the duration of the study.

In order to further validate the proof of concept, the relationship between abstinence during the final 8 weeks of the study and antinicotine Ab concentrations (AUC) is shown in **Figure 2b** for all subjects who received the vaccine. CAR from weeks 45 to 52 are displayed for each 10th-percentile increase in AUC. The proportion of abstinent subjects increased with increasing AUC percentile, and the ordered ranking was maintained.

Effects of high Ab on time to continuous abstinence. An exploratory analysis examined the rate and time to continuous abstinence through to the end of the study (**Figure 2c**). Most smokers quit soon after the TQD, with the high-Ab group showing a clear divergence from both the placebo and low-Ab groups. Among the 18 high-Ab continuous abstainers, 12 initiated abstinence

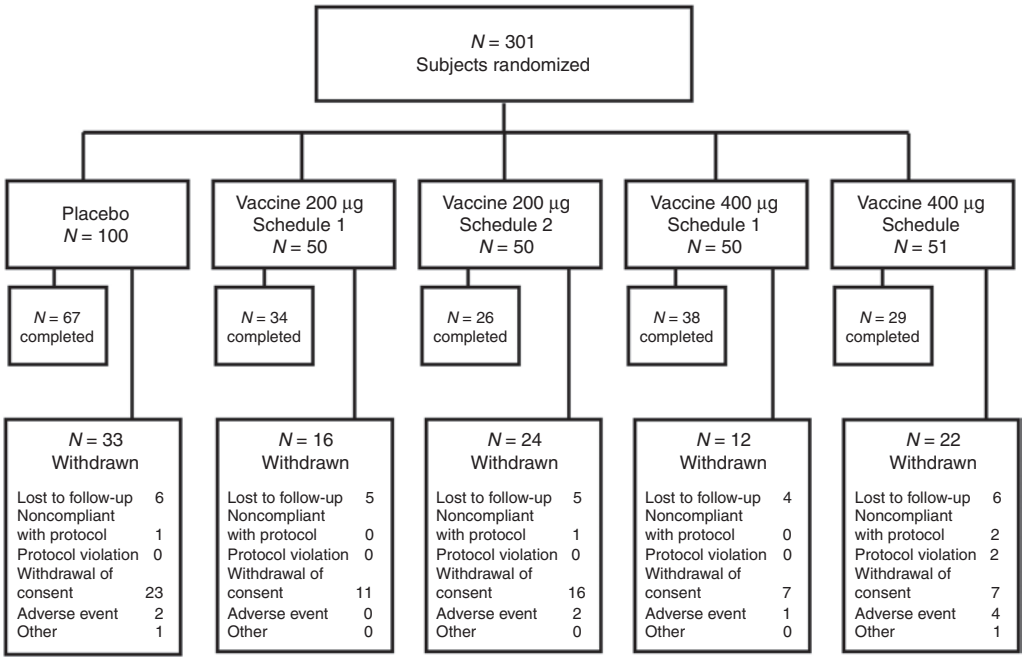

Figure 1 Subject disposition.

prior to the primary end point, and 6 initiated abstinence after the start of the primary end point. Furthermore, 3 of the 15 subjects who were in abstinence during the primary end point had relapsed by the end of the study. Cox proportional hazards analysis demonstrated the superiority of the high-Ab group as compared with the placebo group ($P = 0.0069$, hazard ratio of 2.76).

Evaluation of long-term abstinence. Given that most of the subjects achieved abstinence shortly after their TQD, additional analyses were undertaken to evaluate prolonged abstinence up to 6 and 12 months, after allowing a 2-week grace period after the TQD.[15] Prolonged abstinence is defined as not a single puff during the period from 2 weeks after the TQD for 20 and 44 weeks (6 months and 12 months from initiation of treatment, respectively).

Prolonged abstinence rates to 6 months were significantly higher in the high-Ab group as compared with the placebo group (19.7% vs. 6.0%, $P = 0.0060$, OR = 4.41, 95% CI 1.53–12.71), and there were no significant differences between the results for the placebo and low-Ab groups (7.9% vs. 6.0%, $P = 0.60$). Subjects with high Ab were also significantly more likely to be abstinent for 12 months as compared with those in the placebo group (18.0% vs. 6.0%, $P = 0.014$; OR 3.84; 95% CI 1.32–11.20). The results for the low-Ab group did not differ significantly from those of the placebo group (7.1% vs. 6.0%, $P = 0.67$).

Cigarette smoking in nonquitters. Statistically significant differences were observed with respect to reduction in daily cigarette consumption and in cotinine levels between nonabstainers (weeks 19–52) with high Ab levels and nonabstainers in the placebo group ($P = 0.0015$ and $P = 0.019$, respectively; see **Figure 3a,c**). The difference in the median reduction in cigarette consumption, after the TQD, between the high-Ab group nonabstainers and the placebo group nonabstainers was, on average, 4.6 cigarettes/day. Similarly, geometric mean concentrations of cotinine were 19.0% lower on average after the TQD in the high-Ab nonabstainers as compared with the placebo group nonabstainers. Median cigarettes/day and cotinine geometric mean concentrations for the placebo and low-Ab groups were nearly identical over the study period. There were no differences in mean CO across all three groups (see **Figure 3b**).

Of the total of 301 subjects, 15 subjects had a more than twofold increase in the number of cigarettes smoked per day after the TQD as compared with baseline; there was no significant difference between the placebo group ($n = 4/100$) and the 3′AmNic-rEPA group ($n = 11/201$) in this regard. The highest smoking levels observed after the TQD were fivefold higher than baseline in the placebo group and fourfold higher than baseline in the 3′AmNic-rEPA group. The elevated smoking levels fell below twofold of baseline levels in 11 of the 15 subjects by the end of the study. The number of subjects in whom the smoking levels remained elevated was similar in the 3′AmNic-rEPA group

Table 1 Demographics and smoking history at baseline

		Vaccine 200 µg		Vaccine 400 µg		
Variables	**Placebo** ***n*** **= 100**	**Schedule 1** ***n*** **= 49–50**	**Schedule 2** ***n*** **= 49–50**	**Schedule 1** ***n*** **= 50**	**Schedule 2** ***n*** **= 51**	**Total** ***N*** **= 301**
Age (years)						
Mean ± SD	47 ± 11	50 ± 10	46 ± 11	48 ± 11	48 ± 12	48 ± 11
Gender						
	n **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[b]**
Female	50 (50.0)	26 (52.0)	29 (58.0)	29 (58.0)	24 (47.1)	158 (52.5)
Male	50 (50.0)	24 (48.0)	21 (42.0)	21 (42.0)	27 (52.9)	143 (47.5)
Race						
	n **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[b]**
White	88 (88.0)	47 (94.0)	42 (84.0)	47 (94.0)	47 (92.0)	271 (90.0)
Other	12 (12.0)	3 (6.0)	8 (16.0)	3 (6.0)	4 (8.0)	30 (10.0)
Fagerström total[c]						
Mean ± SD	6.1 ± 1.9	6.3 ± 2.1	5.8 ± 2.0	5.8 ± 2.0	6.6 ± 1.7	6.0 ± 2.0
Median (range)	6 (1–10)	7 (1–10)	6 (1–10)	6 (1–10)	6 (3–10)	6 (1–10)
Cigarettes/day						
Mean ± SD	24.7 ± 8.8	24.8 ± 9.1	22.6 ± 7.0	24.3 ± 9.4	25.6 ± 10.5	24.0 ± 9.0
Median (range)	20 (15–50)	20 (15–50)	20 (15–40)	20 (15–60)	20 (15–70)	20 (15–70)
Previous quit attempts						
	n **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[a]**	***n*** **(%)[b]**
Yes	96 (96.0)	48 (96.0)	47 (94.0)	48 (96.0)	49 (96.1)	288 (95.7)

[a]Percentages based on total number of subjects within treatment group. [b]Percentages based on total number of subjects who received treatment. [c]Fagerström score assesses the severity of nicotine addiction ranging from 0 (minimum) to 10 (maximum).

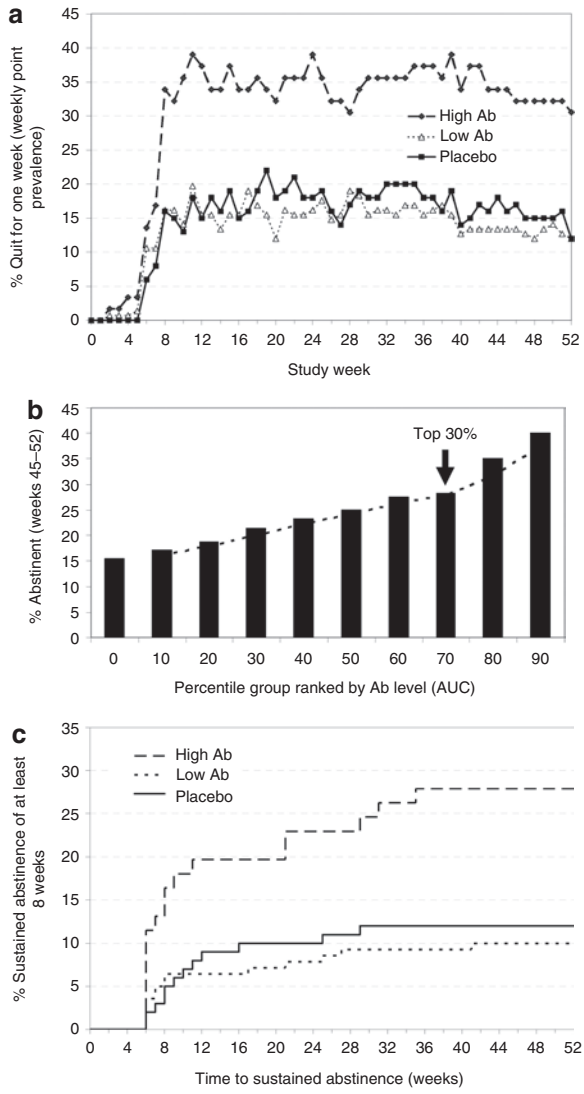

Figure 2 Abstinence rates in intent-to-treat population. (**a**) Seven-day point prevalence abstinence rates for high-antibody (top 30% AUC), low-antibody (bottom 70% AUC), and placebo groups over the course of 52 weeks. (**b**) Percentage of subjects abstinent during weeks 45–52 (8-week continuous abstinence), by AUC. The AUC is displayed in 10th-percentile point increments. (**c**) Time to 8 weeks of sustained abstinence before week 46 and continuous abstinence maintained through to week 52, stratified by group: high-antibody (top 30% AUC), low-antibody (bottom 70% AUC), and placebo. The data from dropouts were censored at week 52. Ab, antibody; AUC, area under the curve.

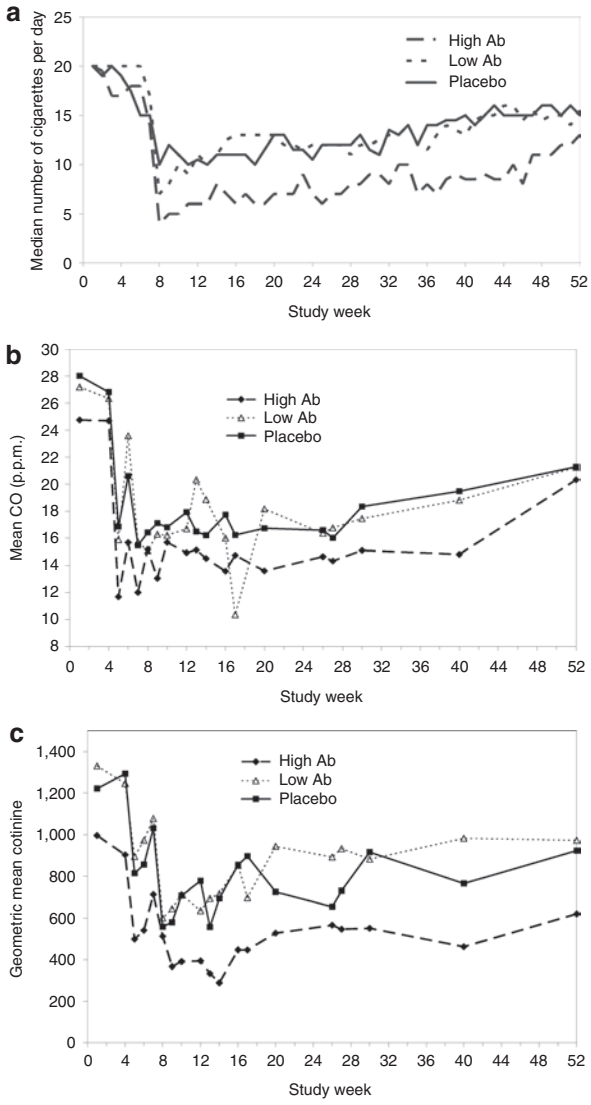

Figure 3 Assessment of smoking in non abstainers. (**a**) Median number of cigarettes per day, (**b**) mean CO levels, and (**c**) geometric mean cotinine levels among subjects who did not abstain from smoking across all three groups: high antibody (top 30% AUC), low antibody (bottom 70% AUC), and placebo. Time, on the *x* axis, is adjusted to align the target quit date between schedule 1 and schedule 2. Ab, antibody; AUC, area under the curve.

(n = 3/201) and in the placebo group (n = 1/100). Individual subjects with CO levels elevated by at least twofold relative to baseline values were also assessed. The results were similar, with no significant differences between the placebo (n = 5/100) and 3′AmNic-rEPA (n = 13/201) groups.

Withdrawal symptoms. No significant intergroup differences were observed in overall withdrawal severity in the three groups ($P > 0.22$).

Effects of dose and schedule

Immunogenicity and efficacy by study group. **Figure 4** depicts immune response by study group from baseline to week 52. Antinicotine Ab geometric mean concentrations increased across all active-treatment groups after each vaccination, with each subsequent dose resulting in a higher Ab response than the previous dose. Schedule 2 resulted in a higher initial increase in Ab concentration. The 400-μg/schedule 2 group demonstrated the highest Ab concentrations. However, no significant differences ($P > 0.05$) were observed in AUC, C_{max}, and C_{avg} across the treatment groups up to 26 weeks and also up to 52 weeks, probably because the study was not powered to detect such differences.

An ITT analysis demonstrated that the schedule 2, 400-μg dose group showed significantly higher prolonged abstinence up to 6 months as compared with the placebo group (17.6% vs. 6.0%; P = 0.015; OR of 4.14; 95% CI 1.32–13.02). However, the difference in abstinence rates was not significant between the

schedule 2, 200-μg dose group and the placebo group (14.0% vs. 6.0%, $P = 0.054$; OR = 3.23; 95% CI 0.98–10.67). Similarly, there was no significant difference in abstinence rates in each of the dosage groups in schedule 1 relative to the placebo group ($P > 0.84$). The schedule 2, 400-μg dose group also showed the highest rates of prolonged abstinence up to 12 months, significantly higher than the placebo group (15.7% vs. 6.0%, $P = 0.038$; OR = 3.44; 95% CI 1.07–11.04). However, the difference was again not significant between the schedule 2, 200-μg dose group and the placebo group (14.0% vs. 6.0%, $P = 0.056$; OR = 3.21; 95% CI 0.97–10.63) and in each of the schedule 1 dosage groups relative to the placebo group ($P > 0.88$).

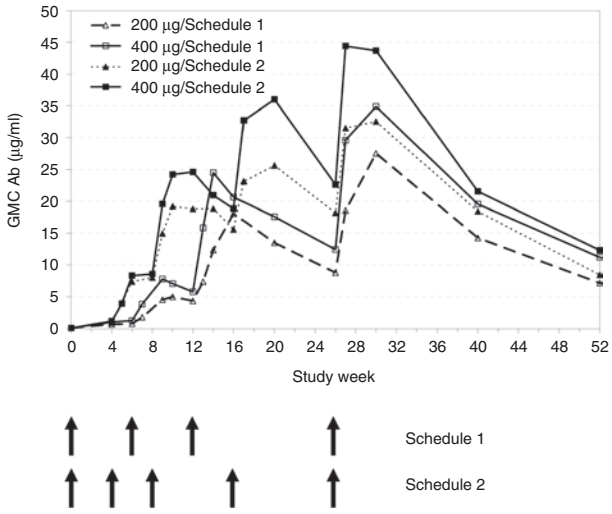

Figure 4 Geometric-mean antibody concentrations (μg/ml) by treatment group. Ab, antibody; GMC, geometric mean concentration.

Safety. **Table 2** shows the number of subjects who experienced local and systemic reactogenicity. Reactogenicity events were aggregated over all injections. Overall, aches and tenderness were the most commonly reported local events, with at least one such report being made by nearly all the subjects (86–98%) in each treatment group. Myalgia, malaise, and headache were the most commonly reported systemic events (64–88% of subjects). Swelling, heat, burning, erythema, and nausea were reported by about half the subjects. Fever and vomiting were less common (4–16%).

A total of 1,184 treatment-emergent adverse events (AEs), predominantly rated mild or moderate, were reported by 266 of the 301 subjects (87.1% of the 3′AmNic-rEPA recipients and 91.0% of placebo recipients). On average, 3.7 and 4.3 events were observed per person in the vaccinated and placebo groups, respectively, including subjects who reported no events. The distribution of 161 physician-determined treatment-related AEs, according to severity and relationship to treatment, was similar for the 3′AmNic-rEPA and placebo arms. Seven 3′AmNic-rEPA recipients (3.5%) and two subjects in the placebo arm (2.0%) withdrew from the study because of AEs.

Of the AEs reported, 18 were in the serious category: 8 events in the 3′AmNic-rEPA treatment groups among 7 subjects (3.5% of the 3′AmNic-rEPA recipients) and 10 events in the placebo group among 5 subjects (5.0% of the placebo recipients). Only one of these serious AEs (anaphylactic reaction in a 3′AmNic-rEPA 400-μg/schedule 2 recipient) was considered by the investigator to be treatment related. This subject, who had a history of urticaria reaction to penicillin and seasonal allergies, experienced difficulty in breathing, throat tightness, facial erythema, and urticaria 70 min after the initial vaccination. The subject was treated with a single injection of subcutaneous epinephrine and diphenhydramine, which resolved the symptoms. Herpes zoster

Table 2 Comparison of the presence of reactogenicity events by treatment group

	Number (%) of subjects by treatment					
		200 μg		400 μg		
	Placebo ($n = 100$)	Schedule 1 ($n = 50$)	Schedule 2 ($n = 50$)	Schedule 1 ($n = 50$)	Schedule 2 ($n = 51$)	*P* value
Local						
Ache	96 (96.0)	48 (96.0)	43 (86.0)	47 (94.0)	49 (96.1)	0.129
Burning	42 (42.0)	23 (46.0)	22 (44.0)	21 (42.0)	23 (45.1)	0.988
Erythema	39 (39.0)	29 (58.0)	23 (46.0)	27 (54.0)	22 (43.1)	0.180
Heat	42 (42.0)	23 (46.0)	25 (50.0)	27 (54.0)	22 (43.1)	0.661
Swelling/induration	60 (60.0)	33 (66.0)	32 (64.0)	33 (66.0)	29 (56.9)	0.827
Tenderness	95 (95.0)	49 (98.0)	44 (88.0)	48 (96.0)	50 (98.0)	0.126
Systemic						
Fever	10 (10.0)	5 (10.0)	2 (4.0)	8 (16.0)	6 (11.8)	0.403
General discomfort/ malaise	79 (79.0)	38 (76.0)	38 (76.0)	42 (84.0)	42 (82.4)	0.803
Headache	67 (67.0)	35 (70.0)	32 (64.0)	35 (70.0)	36 (70.6)	0.945
Myalgia	86 (86.0)	39 (78.0)	41 (82.0)	46 (92.0)	45 (88.2)	0.315
Nausea	44 (44.0)	17 (34.0)	25 (50.0)	21 (42.0)	25 (49.0)	0.501
Vomiting	6 (6.0)	3 (6.0)	2 (4.0)	2 (4.0)	8 (15.7)	0.111

was reported in six subjects. One of these occurred 3–5 h after the first vaccination and therefore could not have been caused by the vaccination. Of the others, four occurred in 3′AmNic-rEPA recipients (2% of the group), and one occurred in the placebo group (1% of the placebo group). In contrast, the related herpes simplex infection was reported in four of the 3′AmNic-rEPA recipients (2% of the group) and three placebo recipients (3% of the placebo group).

DISCUSSION

The results demonstrated the proof of concept that smokers who achieved higher antinicotine Ab concentrations were more likely to quit and remain abstinent from smoking. The high-Ab group demonstrated the highest abstinence rates independent of the time period of ascertainment of status. Similarly, in a separate study conducted by Cornuz and co-workers[13] to test a different nicotine vaccine (nicotine derivative conjugated to a virus-like particle derived from bacteriophage Qβ), *post hoc* analysis showed subjects stratified to the highest-Ab group had a significantly higher quit rate than those in the placebo group. However, unlike our study, which used the ITT population to establish proof of concept, the result reported by Cornuz *et al.*[13] was observed after eliminating about one-third of the subjects either because they had used nicotine replacement therapies during the course of the study or because their Ab titer data were incomplete.

In this 3′AmNic study, subjects in the high-Ab group were observed to have ORs of 4.4 (95% CI 1.5–12.7) and 3.8 (95% CI 1.3–11.2) for prolonged abstinence rates to 6 and 12 months, relative to the placebo group. Although no direct comparisons can be made, these ORs are not unlike ones observed in the meta-analyses conducted for the US Clinical Practice Guideline, in which the reported ORs ranged from 1.5 (95% CI 1.2–1.7) for nicotine gum to 3.1 (95% CI 2.5–3.8) for varenicline at 6 months after the quit date.[3] If the findings from our study are confirmed in larger studies, immunotherapeutics is likely to emerge as an important aid to smoking cessation.

In this study, no significant compensatory smoking, as determined by the number of cigarettes smoked per day, CO levels, and cotinine levels, was observed in response to the presence of antinicotine Abs. This result is consistent with observations from other studies.[12,13] In this study, subjects in the high-Ab group who did not abstain smoked significantly fewer cigarettes (median reduction of ~5 cigarettes/day) and had lower cotinine levels (~20%) than placebo subjects, whereas there were no significant differences in this regard between the low-Ab and placebo groups. However, a small number of subjects across groups (n = 15/301) smoked more than two times the number of cigarettes relative to baseline; there were no significant differences in this regard between the active-treatment groups and the placebo group.

A major challenge for immunotherapeutics is to stimulate high levels of Ab in the vast majority of smokers who are trying to quit. Vaccine dose and frequency have an impact on the Ab levels attained. The five-injection/400-μg dose was associated with the highest Ab response, although this was not statistically significant, possibly because of the small sample size. Importantly, this dose and schedule demonstrated statistically significant improvement in 44-week CAR as compared with placebo. The finding that the four-injection/400-μg dose was not associated with higher abstinence rates demonstrates that a consideration of both dose and schedule of injection are critical to the outcome. In an independent, follow-up immunogenicity study to examine the feasibility of raising the peak Ab levels, a total of 74 subjects received six injections of 400 μg 3′AmNic-rEPA at weeks 0, 4, 8, 12, 16, and 26. In >80% of the subjects who received the six-dose immunization regimen, the target level of Ab (25 μg/ml) was exceeded by week 14. In contrast, only 50% of the subjects receiving five injections of the 400-μg dose achieved this level by week 14 in our study, and only 7% of the subjects attained this level by the TQD. This finding suggests that more frequent injections and a deferment of the quit date may increase treatment success.

In general, 3′AmNic-rEPA was well tolerated. The frequencies of local and systemic vaccine reactogenicity events were similar in the vaccine and placebo groups and similar to those associated with licensed adult vaccines containing Alum adjuvant.[16] The slight increase in cases of herpes zoster observed in the vaccinated vs. placebo group may be spurious; nevertheless, continued monitoring is necessary to determine whether a causal relationship exists. The fact that there was an occurrence of an anaphylactic reaction, albeit in only one subject with a history of drug allergy to penicillin, suggests a need for continued monitoring and follow-up. (In subsequent completed and ongoing clinical studies comparing 3′AmNic-rEPA and placebo, more than 1,800 subjects have received one to six vaccinations with 400 μg 3′AmNic-rEPA or placebo. Only two additional cases of herpes zoster have been observed. Moreover, no additional anaphylactic/anaphylactoid-type serious AEs have been reported to date.)

In summary, results from this study support the concept that high levels of antinicotine Ab are associated with higher rates of abstinence. These findings suggest that vaccines that confer high levels of Ab by the TQD may be more effective. Other future strategies may include examining additional ways to increase Ab levels across all individuals. Nonetheless, this study demonstrates that 3′AmNic-rEPA has significant potential as an aid to smoking cessation and perhaps also to relapse prevention.

METHODS

Study population. Smokers were recruited through advertisement across nine geographically diverse US sites. Potential subjects were screened through telephone interviews and more extensively screened at the first screening visit. Informed consent was obtained from each subject before screening. All the subjects were 18 years of age or older, smoked at least 15 cigarettes/day, had exhaled CO values of >10 p.p.m., reported that they wanted to quit smoking, and were in good general physical and mental health. Exclusion criteria included recent use of any medications or drugs that might have the effect of interfering with immune response or interacting with the vaccine and pharmacotherapies or other treatments for smoking cessation. For female subjects, a negative urine pregnancy test at enrollment and either active use of acceptable birth control methods for the duration of the study or documentation of surgical sterilization were required.

Study design. This phase II study had a randomized double blind, placebo-controlled, parallel-arm trial design (see **Figure 5**). Four

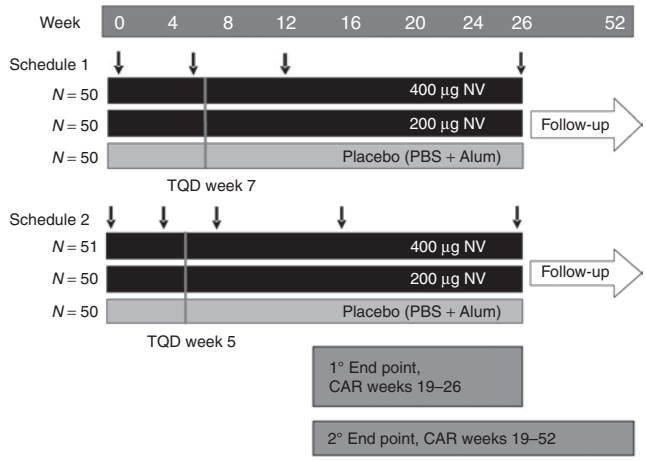

Figure 5 Study design. Arrows denote timings of vaccinations for schedule 1 (weeks 0, 6, 12, and 26) and schedule 2 (weeks 0, 4, 8, 16, and 26). Primary end point (percentage of subjects abstinent between weeks 19 and 26) and secondary end point (percentage of subjects abstinent between weeks 19 and 52) are shown. Alum, aluminum hydroxide adjuvant; CAR, continuous abstinence rates; NV, 3′AmNic-rEPA; PBS, phosphate-buffered saline; TQD, target quit date.

treatment groups received varied doses and/or schedules of intramuscular vaccination: 200 or 400 µg of 3′AmNic-rEPA or placebo according to schedule 1 (weeks 0, 6, 12, and 26) or schedule 2 (weeks 0, 4, 8, 16, and 26). Subjects were randomized within schedule 1 groups ($n = 150$) in a 1:1:1 ratio (200 µg: 400 µg: placebo) and among schedule 2 groups ($n = 151$) in the same ratio. The TQD was set at 1 week after the second injection (end of week 7 for schedule 1 and end of week 5 for schedule 2). If the subjects relapsed (seven consecutive days of smoking) after the quit date, a second quit date coinciding with a future clinic visit was allowed between the time of relapse and week 18. Cessation counseling (based on the USDHHS Clinical Practice Guidelines[17]) for the first quit attempt involved five standardized face-to-face sessions (≤10 min), and for the second quit attempt, a face-to-face session plus 2 postquit telephone counseling sessions.

All the subjects were followed up for 52 weeks after randomization and the administration of the first injection on day 0, for a total of 21 visits. During each injection day, the subjects remained at the study site for 30–60 min for observation after the injection and made a visit 24 h later to enable assessment of side effects. Apart from this, visits ranged from weekly to biweekly and were less frequent after subsequent injections.

Subjects who had not attained abstinence on their TQDs were encouraged to remain in the study and continue to attempt to achieve abstinence. Subjects who left the study were not replaced and were presumed to be smokers for the purposes of analysis.

Institutional review board approval was obtained from all institutions involved in the study. A data and safety monitoring board met four times during the study.

Investigational product. The active investigational product was purified 3′-aminomethylnicotine conjugated to *P. aeruginosa* r-exoprotein A. For the 200- and 400-µg/ml doses, each single-use syringe contained 3′-aminomethylnicotine conjugated to 200 and 400 µg rEPA, respectively, adsorbed to 1.2 mg aluminum hydroxide adjuvant (Alhydrogel 85) in 1 ml phosphate-buffered saline (0.15 mol/l NaCl, 0.002 mol/l $NaPO_4$, pH 7.2, 0.01% polysorbate 80; phosphate-buffered saline). For the placebo dose, phosphate-buffered saline with 1.2 mg Alhydrogel 85 was loaded in a 1-ml single-use syringe.

Measurements. Subjects recorded their cigarette use in an electronic diary every day for 182 days, and then once a week for the remainder of the study. Exhaled CO and urine cotinine were measured at each study visit, except at the visits within 24 h of vaccination. Questionnaires were collected via electronic diary: the Fagerström Test for Nicotine Dependence [18] (administered on days 0, 210, 364), the Minnesota Nicotine Withdrawal Scale[19] (administered weekly until month 6), and data on other tobacco usage.

Serum samples were collected for immunogenicity measurements at 16–17 time points (schedule-dependent) from baseline to week 52. Antinicotine Ab concentrations were measured using enzyme-linked immunosorbent assay.[12] Subjects recorded their local and systemic reactogenicity events for 7 days after each injection. All reactogenicity events were followed until resolution or study completion. Treatment-emergent AEs were recorded for 4 weeks after the last dose, with the exception of serious AEs, for which data were collected up to week 52. The subjects were also periodically monitored at clinic visits for vital signs, weight, hematologic parameters, biochemical parameters, and urinalysis results.

Statistical analysis. The ITT population was used for evaluation of efficacy, safety, and immunogenicity. The ITT population was defined as all subjects who were randomized to treatment.

The primary end point was continuous smoking abstinence for a total of 8 weeks, measured from the beginning of week 19 to the end of week 26 (determined from subject diaries and confirmed by exhaled CO levels of <8 p.p.m.). The analysis for proof of concept involved stratification of the active-treatment recipients into high-Ab responders (top 30% AUC from weeks 0 to 26) and low-Ab responders (bottom 70% AUC from weeks 0 to 26), regardless of the treatment group. An *a priori* decision was made to establish the Ab level cutoff between 50 and 25%. The top 30% by AUC group was selected as the largest group of high-Ab responders between the 25 and 50% levels that demonstrated statistical significance as compared with the subjects receiving placebo. Smoking outcomes were compared by assessing the differences in results between high-Ab subjects and pooled placebo recipients, using logistic regression.

The secondary aims of this study were to assess: (i) the 7-day point prevalence abstinence at various times, CAR during 52 weeks, and time to sustained abstinence (defined as attaining 8 weeks of continuous abstinence at any time before week 46 and maintaining continuous abstinence through 52 weeks; (ii) the impact on compensatory smoking among nonabstainers; (iii) nicotine withdrawal symptoms; and (iv) the immunogenicity, efficacy, and safety of administering either four or five injections of the 200- and 400-µg doses.

For secondary smoking-cessation analyses, logistic regression was used for binary outcomes and Cox proportional hazards regression models and log-rank tests for time to sustained abstinence. Mixed-effects repeated-measures analyses of the number of cigarettes smoked, CO, and cotinine adjusted for baseline were utilized to assess compensatory smoking among nonabstainers and in assessing withdrawal symptoms.

Antinicotine Ab responses were summarized as geometric mean concentration with 95% CIs. Safety was assessed throughout this study primarily in terms of reactogenicity and AEs. Reactogenicity data for 7 days after each injection were tabulated, and the proportions of subjects with any postvaccination reactogenicity, aggregated over all injections, among the five treatment groups were compared using the generalized Cochran–Mantel–Haenszel test.

Any subject who dropped out of the study was presumed to be a smoker for the purposes of analysis. For those who remained in the study, all missing diary data related to cigarette use were imputed utilizing the principle of last observation carried forward. No imputation was used with respect to CO levels. The missing serology data were imputed by first defining a set of injection windows for each schedule. The missing serology data were imputed by using the next available measured serology result in its corresponding window; if the next value was not available, the value of the nearest previous time point in that window was used. The AUC for antinicotine Ab was calculated on the basis of imputed data.

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CONFLICT OF INTEREST

R.C.A., R.E.F.F., P.D.K., M.W.K., and M.N. are employees of Nabi Biopharmaceuticals and have received salary support, stock, and stock options. All other authors were investigators on the clinical trial funded by NIDA and by Nabi Biopharmaceuticals, and some served on an advisory panel. D.E.J. has received research support from Pfizer. D.G. owns shares of Pfizer and has received grant/research support from Pfizer, Addex Pharmaceuticals, Sanofi-Aventis, and GlaxoSmithKline; consulting fees and honoraria from Pfizer, GlaxoSmithKline, and Evotech NeuroSciences; and speaker fees from Pfizer. N.A.R. has received research grant support from Pfizer and is an unpaid consultant to Pfizer and Free & Clear. E.D.G. has received grants from and served as a speaker and consultant for Pfizer. He has also provided advice to or is on the advisory board/panel of Pfizer. He has also served as a speaker for Nabi Biopharmaceuticals. C.A.O. has received grant funding from Pfizer. S.I.R. has participated as a speaker in scientific meetings and courses under the sponsorship of AstraZeneca, GlaxoSmithKline, and Pfizer; has served as consultant for several pharmaceutical companies with relevance to the topics noted in this study (Almiral, Altana, Amersham, Array Biopharma, AstraZeneca, Aventis, Boehringer Ingelheim, Critical Therapeutics, GlaxoSmithKline, Globomax, Intermune, Merck, Novartis, Ono, Otsuka, Roche, Sanofi, Scios, Wyeth); serves on advisory boards of Altana and Pfizer; has been sponsored by GlaxoSmithKline to conduct several clinical trials and received laboratory support; has conducted clinical trials sponsored by Roche, Pfizer, Sanofi, and Novartis; has conducted both clinical trials and basic studies under the sponsorship of Centocor; and has conducted basic studies under the sponsorship of AstraZeneca. This paper was presented in part at the American Heart Association Scientific Sessions 2007, Orlando, FL, 7 November 2007.

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