

Therapeutic vaccines against tobacco addiction

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Most smokers are aware of the dangers of smoking and want to quit, yet few are successful owing to the highly addictive properties of nicotine. Available smoking cessation tools include pharmacotherapies that act in the CNS and show modest long-term efficacy. Additionally, there are emerging concerns that they may cause adverse neuropsychiatric events. Antinicotine vaccines have been used experimentally as aids to smoking cessation. It is hypothesized that antibody capture of nicotine in the bloodstream would prevent it from crossing the blood–brain barrier and reaching the nicotinic receptors. The advantage of the approach includes the relatively gradual rise of antibody levels, which may reduce nicotine withdrawal symptoms, and the possible persistence of the antibodies potentially provides long-term protection, possibly preventing relapse. Proof-of-concept studies of at least two vaccine candidates have shown correlations between antinicotine antibody exposure and smoking abstinence. Unfortunately, the only vaccine tested in two large, randomized Phase III trials, 3'-amino-methyl-nicotine r-exoprotein A conjugate vaccine (NicVAX[®], Nabi Biopharmaceuticals, MD, USA), did not demonstrate efficacy. However, despite the lack of efficacy, there is good reason for continued optimism. This review will summarize the current status of the development of nicotine vaccines, discuss possible causes for the mixed results and review future scientific directions.

KEYWORDS: addiction • antinicotine antibodies • nicotinic receptors • nicotine • NicVAX[®] • therapeutic vaccines • tobacco

Tobacco use & intervention

The dried products of the tobacco plant have been used in the Americas for spiritual, social and medicinal purposes for thousands of years. The first European explorers of the New World helped to widely disseminate the cultivation and use of tobacco during the 16th century. The addictive properties of cigarettes and other tobacco products have been well recognized and are similar to the physical addiction caused by opiates. This is due in part to the interaction of the alkaloid nicotine in tobacco with nicotinic acetylcholine receptors (nAChRs) in the brain.

In the early 20th century, the health consequences of smoking were recognized. Basic and epidemiological studies demonstrated a causal relationship between cigarette smoking, premature death and the occurrence of a number of diseases including lung cancer, obstructive lung disease, infections and heart and vascular disease. Disease related to smoking occurs in nearly all organ systems. Currently, there are approximately 1.5 billion smokers worldwide, and smoking contributes to 5 million deaths

worldwide each year. It is estimated that half of all persistent smokers will die of a tobacco-related illness [1]. The societal cost of smoking is quite high with approximately US\$200 billion annually for healthcare costs and lost productivity in the USA alone.

Reduction in morbidity and mortality due to smoking are the primary goals of tobacco control efforts. Governmental public health initiatives include taxation policies, health consequences warnings and unsightly labeling of cigarette packages in some countries, tobacco control legislation, public education, medical campaigns and provision of tobacco cessation treatments. Successful quitting is more likely to occur with behavioral counseling support and/or pharmacotherapy as opposed to 'cold turkey' attempts. It is encouraging that tobacco use rates have decreased in response to these interventions but have recently reached a plateau in the USA at approximately 20%. Despite the significant progress, morbidity and mortality due to cigarette smoking remain a considerable problem in the developed and developing world. The WHO has set reduction of smoking rates as a worldwide goal.

Generally, individual smoking cessation efforts, consisting of behavioral and/or pharmacological therapies, have shown very modest effects. With such efforts, at 1 year, between 5 and 20% of the subjects are abstinent. Thus, the vast majority of subjects have either failed to quit or have relapsed by 1 year. In addition, a cause for concern for some practitioners and smokers is that several drugs have serious side effects that warranted 'black box' label warnings related to neuropsychiatric events. Since the vast majority of those who attempt to quit will fail, there is a need for improved approaches to smoking cessation. A safe and effective means of attenuating the effects of nicotine would be of major interest in the potential treatment of tobacco abuse.

The authors and others have evaluated the use of active immunotherapy for smoking cessation. It is hypothesized that antinicotine antibody capture of nicotine serves to break the cycle of reward by binding to nicotine and sequestering it in the bloodstream, thus reducing the amount of free nicotine that can cross the blood–brain barrier and reach the nicotinic receptors in the brain. This approach has several theoretical advantages, including the relatively gradual rise of antibodies over several months, which may be less likely to precipitate nicotine withdrawal; the persistence of antibodies for months after discontinuation of vaccination, which potentially provides long-term protection, possibly preventing relapse; and as the antinicotine antibody cannot cross the blood–brain barrier, no direct CNS activity and a lack of CNS-related adverse events are predicted.

Current theories of nicotine addiction

Inhalation of cigarette smoke provides a highly efficient delivery of nicotine to the bloodstream and finally to the brain. Within seconds, nicotine enters into the brain via the pulmonary artery, providing a spike of nicotine concentration and the associated elation feelings. This feeling occurs when nicotine crosses the blood–brain barrier and binds to nAChRs, causing the release of dopamine. The release of dopamine is thought to be a key mechanism in the maintenance of nicotine dependence.

Significantly, nicotine has been demonstrated to upregulate acetylcholine receptor availability in the brains of humans based on single-photon emission computed tomography imaging studies. In comparison with nonsmokers, recently quit smokers (7 days) exhibit significantly higher levels of available β -subunit-containing nAChRs throughout the cerebral cortex (26–36%) and in the striatum (27%) than in nonsmokers [2], thereby sensitizing the recently quit smoker to the effects of nicotine, providing one hypothesis for the physiological basis of the high rate of relapse and the addictive capacity of nicotine. In a similar study, investigators demonstrated that the binding potential of the β 2 nAChRs in the brains of smokers decreased by 33.5% after 4 h of smoking cessation, increased by 25.7% after 10 days of smoking cessation, and decreased to baseline levels (as observed in nonsmoker control subjects) after 21 days of smoking cessation [3]. Interestingly, this time course correlates with the high incidence of relapse within the first weeks of quitting either on or off therapy. Furthermore, smokers who successfully quit using

varenicline relapse at a significant rate following discontinuation of treatment, resulting in an approximately 50% rate of relapse within 1 year of initiating treatment, underscoring the need for a long-acting agent.

Use of vaccines to treat nicotine addiction

Antinicotine vaccines that have been clinically tested are based on conjugate vaccine technology. Generically, such vaccines comprise a nicotine hapten conjugated to a carrier protein (such as a detoxified bacterial toxin) or other immunogenic macromolecule (such as a virus-like particle [VLP]) via a covalent chemical linker. To enhance immunogenicity, an adjuvant is added to the vaccine (such as alum).

The orientation of the nicotine–hapten conjugation-point to the carrier determines the presentation of the hapten to the immune system. Moreover, the linker may sterically impact the conjugate molecule, thereby modulating the specificity of the resulting antinicotine antibodies, which may impact the vaccine efficacy. Several nicotine conjugate vaccines that incorporate a highly immunogenic carrier protein (e.g., bacterial toxoid) or synthetic carrier (e.g., VLP) have been developed and are listed in TABLE 1.

TA-NIC (Xenova/Celtic Pharma, Slough, UK)

The hapten nicotine N1-butyric acid is linked with cholera toxin B carrier protein in TA-NIC. Early clinical studies have indicated that TA-NIC supported smoking cessation although without reporting statistical analyses of the findings [4]. Cholera toxin B has been used in vaccines for cholera and traveler's diarrhea prophylactic vaccines and has demonstrated a favorable safety profile [5,101].

Niccine® (Independent Pharmaceutica, Stockholm, Sweden)

Niccine, another protein conjugate vaccine, is based on tetanus toxoid. Niccine links the IP18 hapten conjugated to tetanus toxoid via the 6-position, forming a carbodiimide bond [6–9].

Nic002 (Cytos/Novartis, Zurich, Switzerland)

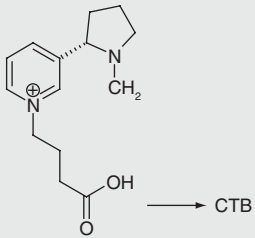

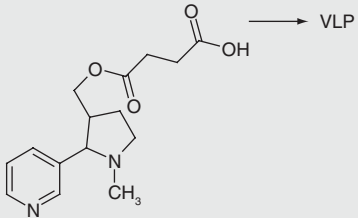
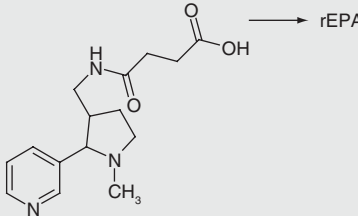
VLP-based conjugate vaccines utilize the synthetic viral particle (capsid) to present nonviral antigens (e.g., nicotine hapten) to the immune system. The synthetic capsid derived from phage Q β is used to present a modified nicotine antigen (hapten) in Nic002. A succinimate linker at the 3' position of the nicotine hapten selected, O-succinyl-3'-hydroxymethylnicotine, is conjugated to the Q β capsid proteins assembled into a VLP [10].

NicVAX® (Nabi Biopharmaceuticals, MD, USA)

The conjugate vaccine NicVAX is the most advanced in development, having completed two Phase III studies [FAHIM R, KESSLER P, KALNIK M ET AL., MANUSCRIPT IN PREPARATION]. NicVAX is conjugated by linking the 3' amino group of 3'-aminomethylnicotine (hapten) with the amino acid side chain and terminal amino groups of *Pseudomonas aeruginosa* exoprotein A (rEPA) via a succinyl linker (3'-AmNic-rEPA).

The recombinant exotoxin carrier protein has been modified by an amino acid deletion to render the molecule nontoxic. The

Table 1. Nicotine vaccines evaluated in humans.

Vaccine	Phase (stage)	Carrier	Adjuvant	Linkage site	Hapten
SEL-068 (Selecta Biosciences, MA, USA)	I (ongoing)	Proprietary nanoparticle technology	T-cell targeting peptide		Nicotine is covalently conjugated to the nanoparticle surface (details not disclosed)
TA-NIC (Xenova/Celtic, Slough, UK)	II (halted)	Recombinant CTB	Alum	N1	
Niccine® (Independent Pharmaceutica, Stockholm, Sweden)	II (halted)	TT	Alum	6	
Nic002 (Cytos/Novartis, Zurich, Switzerland)	II (ongoing)	VLP of bacteriophage Qβ	Alum	3'	
NicVAX® (Nabi/GlaxoSmithKline, London, UK)	III (completed)	<i>Pseudomonas aeruginosa</i> rEPA	Alum (Alhydrogel 85)	3'	

CTB: Cholera Toxin B; rEPA: Recombinant exoprotein A; TT: Tetanus toxoid; VLP: Virus-like particle.

safety of vaccines based on detoxified rEPA has been demonstrated in more than 10,000 volunteers who have been vaccinated with investigational products that target *Staphylococcus aureus* [11] and *Salmonella typhi* [12].

SEL-068 (Selecta Biosciences, MA, USA)

A next-generation nanoparticle vaccine incorporating a targeting moiety for T-cell activation has been recently advanced into human trials [102]. Pittet *et al.* indicate that SEL-068 is “comprised of four main components: a biodegradable and biocompatible polymer matrix, a synthetic TLR agonist, a novel universal T-cell helper peptide and nicotine covalently conjugated to the nanoparticle surface (B cell antigen). The nanoparticles are designed to

flow freely to the lymph nodes, enabling direct and concomitant delivery of all required components to the responsive cells of the immune system” [13].

Preclinical data for each of the vaccines have been recently reviewed [14]. Potential vaccine candidates have been characterized by studies that evaluated antibody response, antibody affinity and specificity and measurement of antibody subclasses. Physiological and behavioral studies were conducted for some of the candidates to demonstrate that immunization or passive transfer of antibody blocks the entry of nicotine into the brain, blocks the effects of nicotine receptor occupancy (dopamine release) or interferes with the physiological effects (blood pressure) or behavioral responses to nicotine (withdrawal symptoms). Peer-reviewed data for some

candidate vaccines are not available; however, in general these vaccines demonstrated robust, highly specific immune responses, with minimal cross reactivity to nicotine metabolites and related molecules, and were shown to interfere with the physiological and behavioral effects of nicotine (TABLE 2). The specificity and high affinity of the antibodies, the reduction in distribution of nicotine to the brain and the physiological effects suggested that vaccination might alter the behavioral responses to inhaled nicotine.

Summary of vaccine trials in smoking cessation in humans

The candidate vaccines mentioned above have all advanced into human clinical studies with mixed results [14]. Proof-of-concept studies of two vaccine candidates indicated that reduction in smoking cessation rates occurs in subjects with robust immune response (approximately the top third of responders) to the vaccine [15,16]. However, one vaccine candidate, NicVAX, failed to demonstrate efficacy in two completed Phase III studies. While

Table 2. Preclinical and clinical evaluation of nicotine vaccines.

Vaccine	Immune response characteristics	Behavioral effects	Physiological actions	Effects in humans	Note	Ref.
TA-NIC (Xenova/Celtic, Slough, UK)	Not reported	Not reported	Not reported	Not reported. Failed to demonstrate efficacy in Phase II proof-of-concept study		[17]
Niccine® (Independent Pharmaceutica, Stockholm, Sweden)	Rat model: nicotine-specific Ab titers 1:10,000, vs undetectable in control animals. Low levels of competition with nicotine and nicotine-N-oxide, no detectable competition observed for cotinine, acetylcholine, noradrenaline, dopamine and serotonin	Immunized animals with high titers did not reinstate nicotine self-administration vs animals with low or no antibody	Immunization with IP18-KLH prevented additional dopamine release	Not reported. Failed to demonstrate efficacy in Phase II relapse prevention trial	KLH carrier was used in preclinical studies	[6–8,17]
Nic002 (Cytos/Novartis, Zurich, Switzerland)	Murine model: peak antibody levels 66 µg/ml (without alum) and 164 µg/ml (with alum) affinity 37 nM (with alum) and 46 nM (without alum). High IgG2a titers (Th1-type immune response). Cotinine and acetylcholine did not compete with titrated nicotine for binding to Ab	Reduced entry of radiolabeled nicotine into the brain, 57% (with alum), 40% (without alum)	None reported	Per-protocol analysis of Phase II study stratified by antibody concentration, continuous abstinence rate at month 6 was 56% in high-antibody group vs 32.1% in placebo		[10,17]
NicVAX® (Nabi/GlaxoSmithKline, London, UK)	Rat model: nicotine-specific IgG 620 µg/ml, antibody affinity was determined to be 10 nM. Antibody 2.7% cross-reactivity to cotinine and <1% cross-reactivity to acetylcholine	Passive transfer of antinicotine IgG or vaccination reduced nicotine transfer into rat brain in acute and chronic nicotine administration models [NABI, DATA ON FILE]	Passive immunization blocked nicotine-induced pharmacological effects (increased blood pressure, increased locomotor activity and increased ACTH secretion) Vaccination blocked the ability of exogenous nicotine to reverse nicotine withdrawal	Abstinence related to antibody titer in Phase II proof-of-concept study. Phase III efficacy not demonstrated (see below) 12% reduction in CNS nicotine binding	Incomplete Freund's adjuvant used in preclinical models	[15,21, 23–25,30]
SEL-068 (Selecta Biosciences, MA, USA)	Robust immune responses generating high-titer and high-affinity antibodies have been reported in mice and nonhuman primates	Not reported	Not reported	Not reported		[13]

Ab: Antibody; ACTH: Adrenocorticotrophic hormone; KLH: Keyhole limpet hemocyanine.

the results are disappointing, the authors and others believe that active immunization for smoking cessation is still a viable strategy if the vaccine immunogenicity (quantity and quality) can be improved.

TA-NIC

No peer-reviewed publications of the results of the preclinical and clinical studies of TA-NIC are available. A proof-of-concept study was conducted between 2006 and 2007 and reported by the company to yield negative results likely due to a manufacturing error [17].

Niccine

The academic/commercial partnership that evaluated Niccine selected a novel paradigm for clinical testing; the investigators focused on relapse prevention instead of smoking cessation. The Phase II study for relapse prevention was conducted in Denmark and Sweden (EudraCT 2007-003250-29). Subjects that quit following treatment with varenicline were randomized 1:1 to receive seven vaccinations monthly with Niccine or placebo [18,103]. Relapse prevention is a novel and challenging clinical trial design (see alternative design tested for NicVAX below). It has been reported that the trial did not meet its primary end point [17], and it is believed that development has been halted.

Nic002

To date, Nic002 (Nic-Q β) has been evaluated in three clinical trials. In an initial study in humans, the safety, tolerability and immunogenicity of the vaccine was tested in 40 nonsmoking volunteers. The vaccine had a favorable safety profile and produced a high level of antibody specific to nicotine [10]. In a Phase II study, 341 adult subjects were randomized (2:1) to receive five vaccinations 1 month apart of alum-adjuvanted 100 μ g of the vaccine (229 subjects) or placebo (112 subjects) [16]. From months 1 to 4, behavioral counseling was provided and subjects were advised to quit at month 1 (second vaccination). The abstinence rates were similar between the vaccine and placebo for the primary end point; the percentage of subjects with carbon monoxide-confirmed abstinence from month 3 to month 6 were 30.1 versus 26.1% for the Nic002 and placebo groups, respectively ($p = 0.44$). When the impact of several variables such as demographics, immunologic and disease severity variables (age, gender, bodyweight, number of cigarettes smoked, duration of smoking, Fagerström's score) were tested using logistical regression, antinicotine antibody titer at month 2 was the only variable that showed a significant influence on abstinence rates in the intention-to-treat population ($p = 0.027$). In the per-protocol analysis, which excluded subjects who concomitantly used nicotine replacement therapy (thought to reduce or interfere with antibody binding capacity) or who were lacking serology data, the continuous abstinence rate from month 2 to 6 was significantly different between the high-antibody responder group versus placebo at 56 versus 32.1%, respectively ($p = 0.004$). Identical abstinence rates (32.1%) were reported for the medium- and low-antibody responder groups, which was also similar to placebo. Significantly, at 12 months,

the abstinence rate was 41.5% in the high-antibody responder group compared with 21.3% in the placebo group ($p = 0.012$).

Influenza-like symptoms and fever were reported in a greater percentage of subjects receiving Nic002 [16] than had been reported for the other nicotine conjugate vaccines [15]. These inflammatory reactions led to a decision to reformulate and to conduct an additional Phase IIb study of Nic002 for smoking cessation using the new formulation. The proof-of-concept, double-blind placebo-controlled study evaluated 200 smokers who wanted to quit smoking. In 2009, it was reported that after an interim analysis, the primary end point had not been met [104]. A clinical imaging study to examine the effect of Nic002 vaccination on nicotine pharmacokinetics and pharmacodynamics is ongoing (ClinicalTrials.gov identifier: NCT01280968 [105]).

NicVAX

NicVAX has been evaluated in humans through Phase III smoking cessation studies (see below). These human studies included immunogenicity to identify an optimized vaccination regimen, as well as smoking cessation studies to test the efficacy of the vaccine compared with placebo.

Six Phase I and II human trials were conducted by Nabi Biopharmaceuticals (MD, USA) prior to initiating a Phase III program. These initial studies evaluated doses of NicVAX ranging from 50 to 400 μ g, each administered up to six times as separate vaccinations over a 26-week period. Intervals between vaccinations for the primary series of vaccinations ranged from 2 to 6 weeks. These immunogenicity studies have been published, in part, elsewhere [19,20].

Overall, the vaccine was well tolerated and local reactogenicity was consistent with that typically reported for intramuscular administration of alum-adjuvanted vaccines, was roughly similar to those reported for alum placebo and was similar to other licensed vaccines that contain alum.

Hatsukami *et al.* reported proof-of-concept for NicVAX [15]. This Phase IIb, double-blinded, placebo-controlled study was designed to assess the proof-of-concept efficacy of NicVAX for smoking cessation. Smokers who wanted to quit ($n = 301$) were randomized to two NicVAX doses (200 and 400 μ g) or placebo, in two dosing schedules (five injections at weeks 0, 4, 8, 16 and 26 or four injections at weeks 0, 6, 12 and 26). The five-vaccination dosing regimen, which was administered at weeks 0, 4, 8, 16 and 26 at a dose of 400 μ g, produced the highest levels of antinicotine antibody. Stratification by antibody response was included in the prespecified primary analysis. The rate of smoking cessation, as well as long-term abstinence, were correlated with the level of antinicotine antibodies elicited by NicVAX. The group with the highest antibody response (defined as the top 30% based on area under the curve for antibody levels) showed 8-week continuous abstinence rates at 6 months (weeks 19–24) of 25% compared with 12% for placebo ($p = 0.024$). This high-antibody group also maintained abstinence at a rate almost three times those of placebo at 12 months (18 vs 6%; $p = 0.014$; odds ratio: 3.84; 95% CI: 1.32–11.20). Importantly, secondary analyses without stratification based on antibody level demonstrated that the

subjects who received 400 µg in the five-dose schedule (the group receiving the greatest amount of NicVAX cumulatively) achieved significantly higher rates of smoking cessation and long-term abstinence than the low-antibody responder and placebo groups. Compensatory smoking or increased withdrawal symptoms were similar between the NicVAX and the placebo groups. Local and systemic reactions to the vaccines were also similar between the vaccine and the placebo treatments, and were mostly mild to moderate in severity and resolved rapidly. A subsequent study (ClinicalTrials.gov identifier: NCT00598325 [105]) evaluated the addition of a sixth injection at week 12 to further increase the antibody levels earlier. In this modified scheme, vaccinations were administered at weeks 0, 4, 8, 12, 16 and 26. The introduction of an additional dose at week 12 elicited levels of antibody at week 14 that were comparable with those seen after the 6-month booster dose in the five-injection schedule with a greater proportion of subjects achieving higher levels of antibody earlier in the trial [14,15,21]. Based on these findings, a vaccination schedule of six injections and a dose of 400 µg of NicVAX was selected to be tested in Phase III efficacy trials.

NicVAX Phase III results

Two nearly identical multicenter, double-blind, placebo-controlled randomized Phase III studies (ClinicalTrials.gov identifiers: NCT00836199 and NCT01102114 [105]) were conducted at clinical centers across the USA. Subjects who were motivated to quit smoking were assigned 1:1 to NicVAX or placebo (~1000 subjects per study). These subjects were in good general health, and were smoking at least ten cigarettes or greater per day. Consented subjects were randomized to receive six vaccinations with NicVAX or placebo (alum alone) over 6 months. Behavioral counseling was administered around the target quit dates, set starting at week 14, corresponding to the time in the study when peak antinicotinic antibody levels are generated (see above). The primary end point of the study was the continuous abstinence rate for 16 weeks (week 37 to week 52) ending at 12 months. Abstinence was evaluated by self-reported cigarette consumption and verified by exhaled carbon monoxide. Secondary end points included the abstinence rate at additional time intervals, safety and immunogenicity, and the effect of NicVAX on withdrawal symptoms, cigarette consumption, smoking satisfaction and nicotine dependency.

NicVAX was well-tolerated with a clinically acceptable safety and tolerability profile. However, as indicated above, unfortunately both studies failed to demonstrate efficacy. The abstinence rates in both the NicVAX group and placebo group were identical (11%), which was unexpected based on the encouraging results in the Phase IIB study [22]. A few hypotheses to try to explain the failure are included in the next section.

Possible reasons for nicotine vaccines failure to demonstrate efficacy?

The authors hypothesize that the failure of the Phase III NicVAX studies may be due to: the levels, affinities and specificity of antinicotinic antibody needed to support smoking cessation in a

majority of the smoking population are much higher than that elicited by NicVAX; loss of motivation to quit, due to the delayed target quit date in the vaccine study (more than 3 months as opposed to 1–2 weeks for existing pharmacotherapies), may have led to fewer successful quit attempts by participating subjects.

Significant efforts are aimed at understanding why the current generation of vaccines has failed to provide clinical benefit. Imaging studies have focused on demonstrating in humans the preclinical findings of robust alteration of nicotine kinetics and the blocking of nicotine-induced physiologic changes observed in animals. A recent report examined $\beta 2^*$ -nAChR occupancy by nicotine after vaccination with NicVAX [23]. The preliminary findings indicated a significant reduction in nicotine binding (12%; $n = 11$) in the brain of subjects after immunization. Moreover, plasma nicotine levels within the first 60 min of receiving a dose of nicotine are up to twofold higher in immunized subjects (23 ng/ml nicotine plasma level) as compared with the same subjects prior to immunization (9 ng/ml level). These preliminary studies suggest that the nicotine vaccine is increasing the sequestration of nicotine in the bloodstream along with reduced nicotine binding in the brain, confirming the preclinical studies with this vaccine [10,24,25] [NABI BIOPHARMACEUTICALS, DATA ON FILE]. Larger clinical studies will be needed to confirm these changes in nicotine kinetics and distribution.

Researchers at Duke University (NC, USA) are conducting such a study in 65 smokers. The focus of the study is to test the hypothesis that antinicotinic antibodies elicited by vaccination with Nic002 may change the kinetics of brain nicotine accumulation and distribution of nicotine between the brain and other body tissues (ClinicalTrials.gov identifier: NCT01280968 [105]).

Furthermore, a relapse-prevention study that combines NicVAX and varenicline (ClinicalTrials.gov identifier: NCT00995033 [105]) is testing the effect of an early quit date by combining the vaccine with a rapid-onset pharmacotherapy that supports quit attempts within a few weeks of study entry.

Future directions

Nicotine addiction comprises a combination of pharmacologic, behavioral and environmental influences. Current thinking is that the first generation of nicotine conjugate vaccines is inducing insufficient levels of high-quality antinicotinic antibodies to support smoking cessation as a monotherapy. New technologies are needed to improve the induction of high-quality antinicotinic antibodies.

New adjuvant formulations

The inclusion of novel adjuvant technologies and immunologic targeting are two of the main approaches being researched today. GlaxoSmithKline and Nabi Biopharmaceuticals are evaluating second-generation vaccine candidates by combining NicVAX technology with undisclosed, proprietary GlaxoSmithKline adjuvant technologies.

Pfizer (NY, USA) is in preclinical development with its own nicotine protein conjugate vaccine, NIC7-DT, formulated with a novel CpG adjuvant and has reported that it induces antinicotinic

antibodies of higher titer, avidity and function as compared with a Nic002 mimetic in mice [26].

Next-generation vaccines: beyond protein-carrier conjugates

SEL-068 (Selecta Biosciences, MA, USA) described above is a next-generation nanoparticle-based nicotine vaccine that has been designed to incorporate immune targeting (T-cell helper peptide) and a controlled release Toll-like receptor agonist in the nanoparticle. Robust immune responses generating high-titer and high-affinity antibodies have been reported in mice and nonhuman primates [15]. In November 2011, SEL-068 entered Phase I studies in healthy volunteers to assess the safety and dose-dependence of immune response [102].

A novel gene therapy-based approach has been developed at Cornell University (NY, USA). Preclinical studies conducted in mice demonstrated that a single administration of an adeno-associated virus gene transfer vector expressing high levels of an antinicotine antibody reduced brain nicotine levels to 15% of nonimmunized controls, increased plasma nicotine levels sevenfold and blocked the physiologic effects of nicotine. Additionally, high titers were maintained from a single dose for 18 weeks [27]. If these results translate into humans with a good safety profile, the implications are very intriguing for the potential of a single-administration and long-lasting therapy for the prevention of nicotine addiction.

Combination therapies

Combinations of pharmacological agents are commonly utilized in clinical practice to treat many hard-to-treat diseases such as malignancies, HIV, refractory hypertension and diabetes. These combination strategies rely on using therapeutic agents with differing mechanisms of action. For smoking cessation, the combination therapy of multiple nicotine replacement agents or the combination of nicotine replacement therapy with bupropion have been tested and have demonstrated improved abstinence rates compared with monotherapy. This suggests that the combinations of a short-acting pharmacotherapy with its high relapse rates and neuropsychological side effects and a nicotine vaccine monotherapy with its shortcomings, particularly the prolonged delay until first quit attempt, would be worthwhile to test clinically.

NicVAX-varenicline combination

A combination study (Nabi-4508) was designed to reduce some of the challenges encountered in vaccine monotherapy cessation studies by leveraging the strengths of the rapid onset of action of the potent nicotine receptor partial agonist varenicline and the potential long-term protective effects of antinicotine antibodies arising from vaccination with NicVAX. This alternative approach uses varenicline to induce smoking cessation with an early target quit date, within 1 week of starting varenicline (weeks 2–9), coinciding with multiple vaccinations of NicVAX, with the buildup of antinicotine antibodies reaching peak levels when varenicline treatment is discontinued (FIGURE 1). The alignment of peak antibodies with discontinuation of varenicline is

thought to be important since the proportion of abstainers who relapse in the first 3 months following discontinuation of varenicline monotherapy (weeks 12–24) is approximately twice that of the subjects who relapse in the latter 6 months (weeks 25–52) derived from [28,29].

The combination study design overcomes one of the potential weakness of antinicotine vaccines monotherapy, namely the impact of the delayed (week 14) target quit date as was the case in the NicVAX Phase III studies, as necessitated by the long time needed for antinicotine antibodies to reach high levels. Another key advantage of the combination therapy is that the amount of antinicotine antibodies needed to capture residual nicotine in recently quit smokers on varenicline would be significantly less than needed for an active smoker seeking primary abstinence. The top-line results of the NicVAX-varenicline combination study have been announced in a press release [106] and indicated that the study did not achieve the primary end point and that the combination was not more effective than the varenicline monotherapy. This is yet another disappointing result for therapeutic vaccines as aids for smoking cessation. No technical information has been published yet to discern the reason for such a result but one may speculate that it may be related to suboptimal immune response and/or the quality of the antinicotine antibodies.

Conclusion

Nicotine addiction continues to be one of the largest public health issues facing the world today. Despite decades of research to identify an effective therapy for smoking cessation, current therapies, such as behavioral counseling, nicotine replacement therapies, bupropion and varenicline, are only modestly effective for short periods and the vast majority of those who abstain relapse shortly after finishing the course of therapy. Moreover, pharmacotherapies such as bupropion and varenicline have the additional risk of serious adverse effects, which has led to boxed warnings that has limited their use. Clearly, more effective therapies for long-term abstinence are needed.

Therapeutic vaccines have been investigated for over 10 years as a potentially effective long-term therapy for smoking cessation. Preclinical studies in animal models for smoking cessation as well as early clinical studies have shown promise. The hypothesis is that antinicotine antibodies bind the nicotine from smoking and, because of the size of the resultant molecule, physically prevent the nicotine from crossing the blood-brain barrier and attaching to the nicotinic receptors. In Phase II studies, at least two vaccines (Nic002 and NicVAX) have demonstrated a strong correlation between high antinicotine antibodies and long-term abstinence in prespecified and *post-hoc* stratification analyses.

While several laboratories have developed multiple versions of nicotine vaccines, it is yet to be proven that such vaccines are effective for smoking cessation in large Phase III studies. Indeed, one vaccine (NicVAX), which has been tested in two well-conducted, placebo-controlled Phase III studies, showed no significant difference compared to placebo. It is conceivable that the level of antinicotine antibodies elicited by the vaccine were

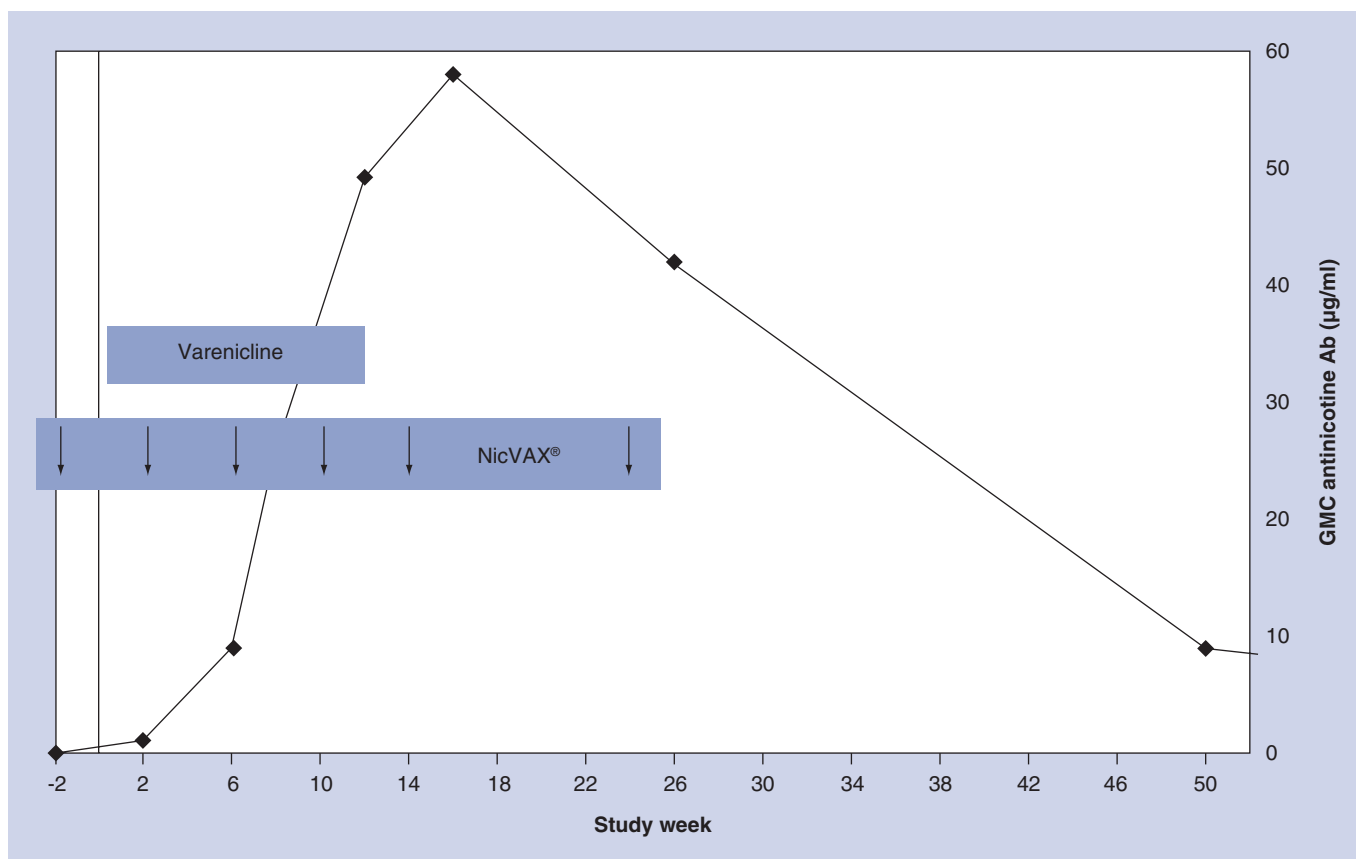


Figure 1. The antinicotine antibody kinetics from a Phase II study of the six-dose regimen of NicVAX® with an overlay of the NicVAX–varenicline combination study (Nabi-4508) dosing regimen. In the ongoing study Nabi-4508, NicVAX was administered six times over 6 months (starting at week 2) and varenicline was dosed 0.5 mg daily beginning at day 0 for 3 days, then 0.5 mg twice-daily for 4 days, followed by 1 mg twice-daily for 11 weeks (starting at week 0). Target quit date for varenicline was weeks 2–9.

Ab: Antibody; GMC: Geometric mean concentration.

Data taken from [NABI-4513, UNPUBLISHED DATA].

not sufficient or of high-enough affinity to bind the majority of the circulating nicotine. It may also be that the length of time needed for sufficient production of antinicotine antibodies is too long and during this long ‘wait period’ smokers may lose their will to quit due to the strong behavioral and psychological aspects of addiction. Nevertheless, enough evidence exists to support continued research in nicotine vaccines as aids to smoking cessation and relapse prevention, in combination with other therapies such as behavioral counseling and possibly with pharmacotherapies. It may also be that new vaccine formulations with more potent adjuvants and/or carriers are needed to ensure the production of high levels of high-quality antinicotine antibodies.

Expert commentary

Smoking cessation has been a formidable challenge due to the high addictive nature of nicotine. Current therapies are not very effective in the long term. New approaches such as therapeutic vaccines have a significant appeal since antinicotine antibodies last for a long period after the end of therapeutic immunization. However, thus far this approach has been disappointing when

traditional conjugate vaccine approaches were employed. Four different vaccines have been tested in clinical trials and after early encouraging clinical trials, all four failed to show efficacy. Notably, the most advanced vaccine, NicVAX, showed no efficacy versus placebo in two well-controlled double-blinded Phase III studies. However, most experts are still optimistic that therapeutic vaccines may yet have a role as aids to smoking cessation.

Five-year view

Despite early setbacks, experts believe that therapeutic vaccines may still be useful as aids to smoking cessation if more powerful adjuvants, other more effective presentations, such as nanoparticle vaccines and/or the use of more immunogenic carrier proteins, are used. Additionally, most experts believe that such vaccines may be more effective as one component in a combination approach, even though initial study results have been disappointing. It is believed that other, more novel approaches, such as gene therapy to stimulate the production of antinicotine antibodies, are much earlier in development and, if successful, are not expected to be available in the next 5 years.

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Key issues

- Smoking cessation remains a significant health-related concern and current therapies are only moderately effective in the short term. It is believed that the highly addictive nature of nicotine is at least partly to blame.
- Therapeutic vaccines against nicotine generated encouraging preclinical and early clinical results. It is believed that the mechanism of action is that the vaccine, when injected, generates antinicotinic antibodies; the latter would capture the inhaled nicotine from cigarettes, sequester it in the blood stream and prevent it from crossing the blood–brain barrier and reaching the nicotinic receptors in the brain.
- Four vaccines have completed later-stage clinical trials. Disappointingly, all four vaccines failed to demonstrate efficacy in humans.
- Future directions are focusing on stronger adjuvants, novel presentations such as nanoparticles and/or more immunogenic carrier proteins. Like other difficult-to-treat diseases, it is also believed that combination therapies, which include vaccines, may be more effective, although recent data of a combination trial using a therapeutic vaccine in combination with varenicline were not more effective than varenicline monotherapy.

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